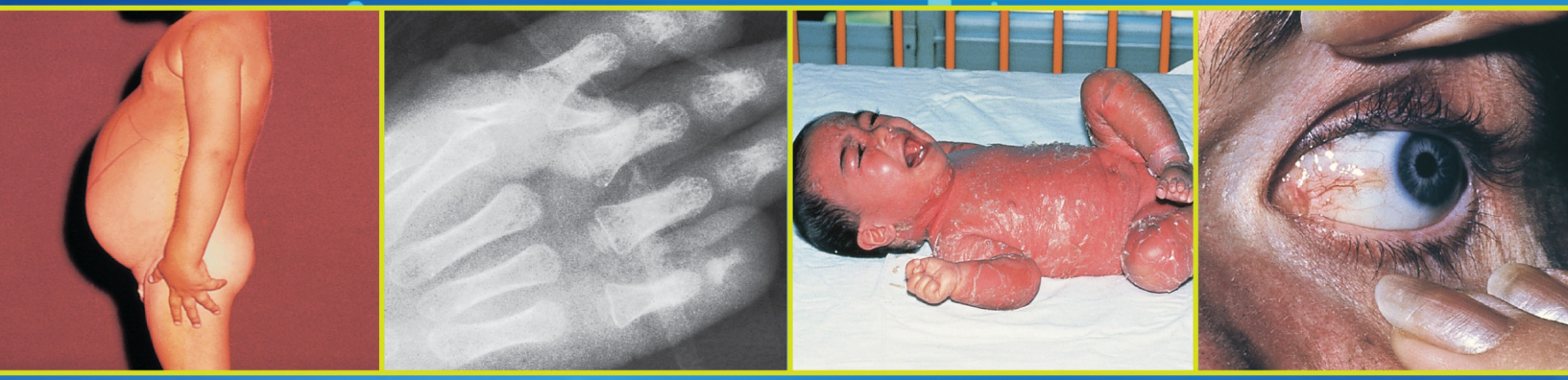
ATLAS OF INHERITED METABOLIC DISEASES

Fourth Edition

William L. Nyhan and Georg F. Hoffmann

With contributions from Aida I. Al-Aqeel and Bruce A. Barshop



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ATLAS OF INHERITED METABOLIC DISEASES

FOURTH EDITION

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Preface

This book is designed as a source of practical information of use in the diagnosis and management of patients with inherited diseases of metabolism. We have kept the focus, as did Garrod, on the inborn errors. This permits a unity of theme. At the same time, the reality is that genetically determined human variation in metabolism leads to an enormous variety of clinical expression crossing most of the boundaries of clinical subspecialty.

We want this book to be helpful to physicians at the bedside, in the intensive care unit, and in the clinics and offices, as well as to biochemical geneticists and clinical chemists involved in laboratory diagnosis. The atlas format has permitted us to include very many illustrations of patients. Metabolic pathways have been shown with a reductionist or high-power view of just that area most relevant to each disease. In addition, the chapters deal with individual diseases. There are introductory chapters to the organic acidemias, the disorders of the urea cycle, the disorders of fatty acid oxidation, the lactic acidemias, the glycogenoses, and the mucopolysaccharidoses, which provide some general considerations of these areas of metabolism and permit us to avoid some redundancy. With these exceptions, each chapter represents defective activity of a single enzyme. Mutations in a single gene can lead to a very large family of different variant enzymes and, accordingly, very different clinical phenotypes. In general, we have considered this variation in each chapter, with emphasis on the most common expression. In two instances, we have given variants separate treatments. There is historical precedent for separate consideration of Hurler disease from the Scheie and Hurler-Scheie variants and for the separate consideration of mucolipidoses II and III. We have continued that. In contrast, we now have an integrated chapter for HPRT deficiency.

The rates of discovery of new or previously unrecognized diseases in this field are enormous. In the 1980s, we saw for the first time, descriptions of many of the currently known disorders of fatty acid oxidation; in the 1990s, we saw the numbers of known discrete mitochondrial DNA mutations increase rapidly. Some of these diseases are turning out to be relatively common. Medium-chain acyl CoA dehydrogenase (MCAD) deficiency occurs once in approximately 10,000 births, and most patients have the same mutation. On the other hand, though it is clear that in the aggregate the inherited diseases of metabolism make up a sizeable portion of human morbidity and mortality, each individual disease tends to be rarely encountered. Even an expert may find years have elapsed since he last saw a patient with a given disorder, reviewed the literature, and ordered it in a way that would help with diagnosis or treatment. It helps to have the relevant information in one place for ready retrieval. This atlas serves that purpose for us. We are hopeful that it will do the same for our readers.

The advent of molecular biologic approaches to genetics and the increasing exploration of the human genome have changed forever the scope of human genetics and the manner in which it is practiced. In the atlas, we have endeavored to seek a balance among the molecular biology and the nature of mutation, the enzymology and intermediary metabolism, and clinical practice. Our focus is on the clinician. Algorithms are provided for the logical work up of a patient with lactic acidemia and disorders of fatty acid oxidation, and a systematic approach to the diagnosis of a patient with hyperammonemia.

Medical genetics is now officially recognized in many countries among clinical and laboratory specialties. Trainees preparing themselves for board examinations might want to read the atlas from cover to cover. We hope that in addition to medical geneticists, pediatricians, neurologists, internists, pathologists, and all those who interact with patients with these disorders will find the atlas of assistance in their practices.

The field is moving so rapidly it is an experience to keep current in any disease. There is much in this book that is new, different, or virtually unique. Certainly, the pictures are for us a resource. Mutations have now been identified in the genes for the very strange ethylmalonic aciduria whose petechial exacerbations lead regularly to treatment for meningococcemia. The discovery of this gene, *ETHE1*, by homozygosity mapping, illustrates the powerful new influence of molecular biology and the data provided by the human genome project in this field.

In I-cell disease and pseudohurler polydystrophy, the basic defect is in the processing of lysosomal enzymes to permit their recognition and entry into cellular lysosomes. The fascinating and novel mechanism uncovered in the multiple sulfatase deficiency defect is in an enzyme which catalyzes a post-translational change of a cysteine moiety in each of the sulfatase enzymes to an amino-oxopropionic

acid moiety, which change normally converts inactive sulfatase proteins to catalytically active enzymes.

Among the challenges for diagnosis and management highlighted in this volume are the disorders of fatty acid oxidation and the lactic acidemias and mitochondrial disease. The latter include the acronymic disorders resulting from mitochondrial DNA mutation and the Pearson syndrome, which may present in infancy as a pure hematologic disorder. It also includes the deficiency of DNA polymerase, which results in a mitochondrial DNA depletion syndrome. The disorders of creatine synthesis are a challenge for diagnosis. They are sometimes suspected when the urine is analyzed for organic acids and amino acids, and everything is high, because we base our analyses per mole of creatinine. They may be elegantly demonstrated by nuclear magnetic resonance spectroscopy (NMRS). It is turning out that these disorders in aggregate are as common as PKU and should be looked for in patients with nonspecific developmental delay.

The atlas was generated by our experience with patients with metabolic disease. We are grateful to the many physicians who have referred these patients to us and to those who have shared their illustrations with us. We are appreciative of the help of many of our fellows and colleagues who have helped us care for and study these patients. They include Drs Nadia Sakati, Richard Hass, Fred Levine, Robert Naviaux, Jon Wolff, Mary Willis, Zarzuela Zolkipli, Ilya Gertsman, and Karen McGowan.

Original artwork was provided by Ms. Michelle Williamson, of BioMedical Design. Images of tandem mass spectrometry were recovered by Mr. Jon Gangoiti of the Biochemical genetics Laboratory at UCSD. We are particularly indebted to the work of Susan Allen, for the conversion of handwritten pages into polished typed electronic manuscripts.

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Preface

> We must not cease from exploration, and at the end of all our exploring, will be to arrive where we started, and know the place for the first time.
>
> *T.S. Elliot, Four Quartets, 1942*

In 1942, only a handful of inborn errors of metabolism, sometimes called orphan disease, were recognized and little or no treatment was available. Genetic counseling was virtually all that was available. Phenylketonuria was then shown by Horst Bickel from the Children's Hospital, Heidelberg, Germany, to be a treatable "genetic" disease in which early diagnosis and dietary treatment prevented impaired mental development. Subsequently, many other inborn errors of metabolism became manageable in a similar way, i.e. with substrate deprivation strategies: maple syrup urine disease, galactosemia, fructosemia, tyrosinemia type 2, and others. Pharmacologic doses of vitamins proved useful in defects of cobalamin and biotin metabolism in distinct forms of homocystinuria, and some others. Avoidance of fasting was recognized as the cornerstone of successful therapy for defects of fatty acid oxidation, treatment has begun to explode over the last decennium as current progress has been made in understanding the molecular and pathophysiologic bases of inborn errors of metabolism. New treatment protocols – new therapeutic agents (drugs and foods) have been described, as has tissue transplantation, enzyme replacement and gene therapy.

The world health organization (WHO), as well as the European Union (EU), have announced genetic and orphan diseases as a major health challenge of the future. The more than 500 inborn errors of metabolism are especially important because of their relatively high frequency and because successful rationale therapy is already available or will become in the near future. As a group, they account for approximately 1 in 100 births worldwide. Scientific and technological advances offer enormous benefit to patients suffering from inborn errors of metabolism often completely preventing life-long burden and suffering. Early diagnosis by extended newborn screening with subsequent early treatment is the most successful approach. Tandem mass spectrometry has recently been implemented in newborn screening programs across an increasing number of countries, and other diagnostic high-throughput techniques including primary molecular diagnostics are at the edge. Handicap and suffering can be prevented for thousands of children and their families. Extended newborn screening is far more cost-effective. The costs of screening programs are greatly outnumbered by the costs for direct health and social costs in childhood.

The field of inborn errors of metabolism is continuing to increase, both in its size and fortunately even more by our knowledge. Clinical expertise and good cooperation between the referring physician and the metabolic specialist and a broad spectrum of metabolic investigations in the center are the keys to successful diagnosis and treatment. Each disease and each patient are different. When molecular genetics came to medicine, there was a widely held belief, that knowing the genotype at the particular locus would predict the corresponding phenotype and assist counseling and treatment. Though genotype-phenotype correlation is strong in some diseases, there is a huge number of examples were the phenotype cannot be explained by the mutations found. It has become obvious that, in addition to mutations of the affected gene and environment, many other factors influence the phenotype. The role of numerous factors affecting post-transcriptional events and their mutual relationships are at best partly understood.

The "Atlas of Inherited Metabolic Diseases" is now set in its fourth edition and has become the in-depth clinical reference resource for inborn errors of metabolism, combining in great detail clinical presentation, treatment, monitoring and course. Following brief but solid biochemical and molecular background information, physicians will find the most comprehensive clinical reference book, with instructive descriptions of clinical situations and the possibility of a visual double check on a metabolic syndrome with physical characteristics through photos found nowhere else. The content of this book draws from decades-long clinical experiences, always asking what could have been done better. It has been, and will continue to be, an invaluable source for metabolic physicians in the care for their patients. Reflecting their experiences in the detail and advice found in the "Atlas of Inherited Metabolic Diseases" they may often find themselves remembering the beautiful lines of T.S. Elliot.

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PART 1

ORGANIC ACIDEMIAS

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1

Introduction to the organic acidemias

The inborn errors of organic acid metabolism represent a spectrum of disorders, most of them relatively recently recognized. Many of them produce life-threatening illness very early in life. They should be suspected in any patient with metabolic acidosis, and certainly when there is an anion gap (Table 1.1). The variety of metabolic pathways involved is indicated in Figure 1.1.

The classic presentation of the organic acidemias is in infancy, often in the neonatal period, followed by recurrent episodes of metabolic decompensation, usually precipitated by infection. The infant begins vomiting and becomes anorexic. This may be followed by the rapid deep breathing of acidosis. A ketotic odor may be appreciated. There may be rapid progression through lethargy to coma, or there may be convulsions. Hypothermia may be the only manifestation besides failure to feed and lethargy. Further progression is to apnea and, in the absence of intubation and assisted ventilation, death.

Initial laboratory evaluation involves tests that are readily available in most clinical chemistry laboratories. Most important in early discrimination are the electrolytes and the ammonia. Blood gases are often the first data available in a very sick infant. Acidosis and hyperammonemia are indicative of an organic acidemia. In contrast, a patient with a urea cycle defect has hyperammonemia and alkalosis. It is important not to delay treatment of acidosis in the belief that the problem is a urea cycle defect. Hyperammonemia regardless of cause must be treated. Hypocalcemia may be a nonspecific harbinger of metabolic disease. Elevated levels of lactate in the absence of cardiac disease, shock or hypoxemia are often seen in organic acidemias as well as in the lactic acidemias of mitochondrial disease. The blood count is useful in indicating the presence or absence of infection. More important, neutropenia with or without thrombocytopenia or even with pancytopenia is characteristic of organic acidemia.

Table 1.1 Mnemonic for the differential diagnosis of metabolic acidosis with an elevated anion gap (DIMPLES)

D	Diabetic ketoacidosis
I	Inborn error of metabolism, iron, isoniazid
M	Methanol, metformin
P	Paraldehyde, phenformin
L	Lactic acidemia
E	Ethanol, ethylene glycol
S	Salicylates, solvents, strychnine

The mnemonic has been written as "mudpiles" or "mudpies", including u for uremia, but, in clinical practice, uremia tends to be recognized as early as the acidosis, making this unnecessary; the latter form leaves out lactic acidemia, an important omission. The current form highlights metabolic causes of acidosis.

In the presence of acidosis suggesting organic aciduria, the assays of choice are organic acid analysis of the urine and acylcarnitine profile of the plasma.

A number of the organic acid disorders are on the catabolic pathways for the branched-chain amino acids, or other amino acids, but the site of the enzymatic defect is sufficiently removed from the step at which the amino group is lost so that the amino acids do not accumulate, and thus these disorders are not detected by methods of amino acid analysis. They remained largely unrecognized until the development of methods of detection, particularly gas chromatography-mass spectrometry (GCMS) [1], that were of sufficient generality not to depend on a single functional group for detection. Quantitative organic analysis is an important aspect of this methodology. Tandem mass spectrometry (MS/MS) [2, 3] (Table 1.2) has added another important method of detection of organic acids as their carnitine esters; this methodology has made these diseases subjects for neonatal screening.

GCMS has been the basis for monitoring levels of relevant metabolites in the course of management. Therapeutic interventions, including cofactor or other dosage and dietary restriction, are dependent on accurate knowledge of the concentrations of those compounds that accumulate behind the block. MS/MS may also serve this purpose. In general, therapeutic efficacy is best when concentrations of accumulated metabolite(s) are kept at the lowest achievable level. This is seldom zero except in cofactor responsive inborn errors, such as biotin-responsive multiple carboxylase deficiency (Chapters 6 and 7). More commonly, a plateau level of metabolite is achieved, at which further restriction of metabolite intake leads to catabolism and an increase in metabolite accumulation, as well as

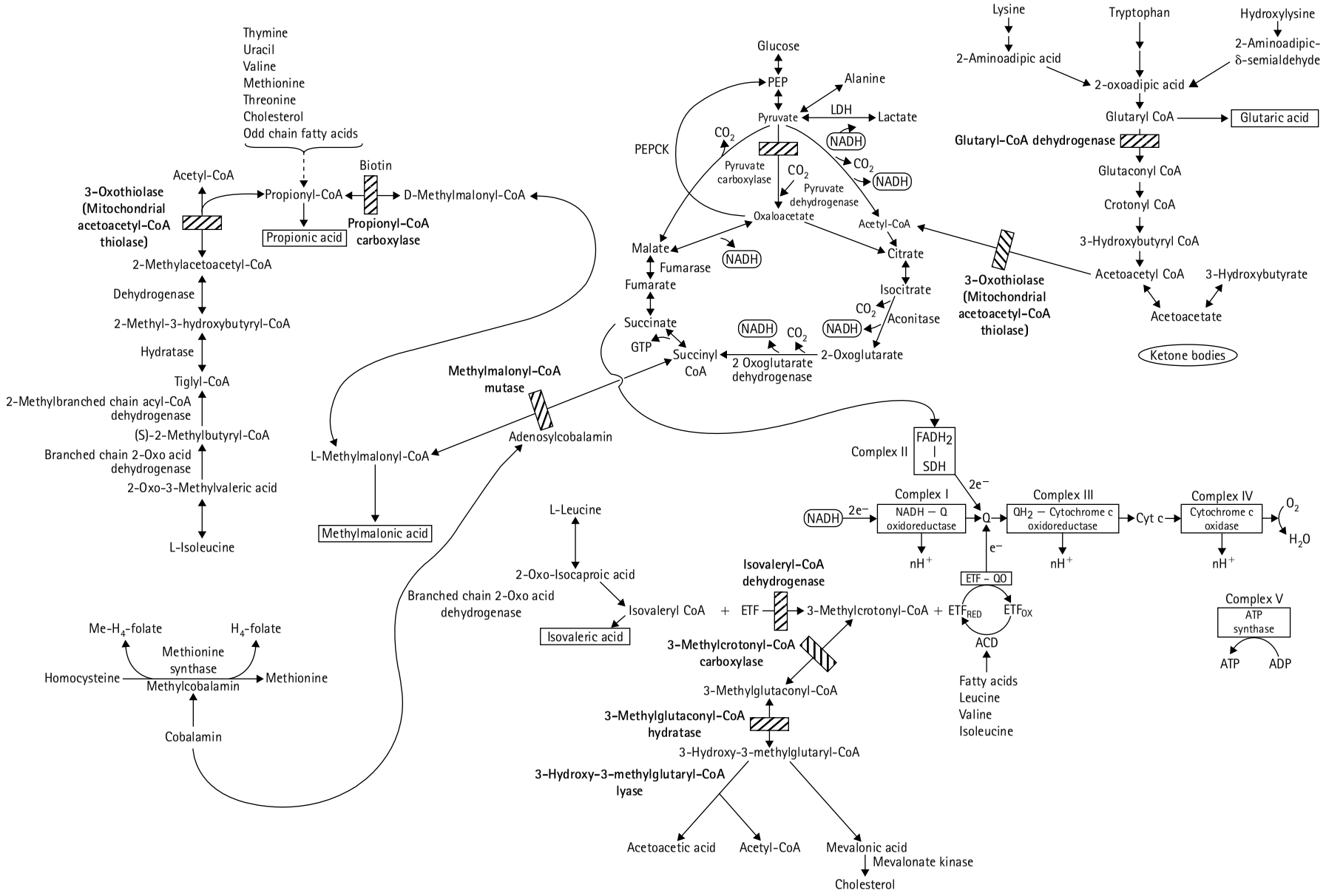

Figure 1.1 Metabolic interrelations of relevance to the organic acidemias. Many of these disorders are characterized by the accumulation of CoA esters. Many present with lactic acidemia.

Table 1.2 Acylcarnitine profiles of plasma in the diagnosis of organic acidemias

Disorder	Acylcarnitine	Control reference[a]	Patient
Propionic acidemia	C3	0.07–1.77	6.50–60.10
Methylmalonic acidemia	C3	0.07–1.77	13.00–90.50
	C4DC	0.00–0.04	0.12–0.94
Ethylmalonic encephalopathy	C4, C5, C4/C3, C5/C3, C2		
Isobutyryl-CoA dehydrogenase deficiency	C4	0.06–1.05	
Malonic aciduria (malonylCoA decarboxylase deficiency)	C3DC	0.06–1.05	
2-Oxothiolase deficiency	C5:1	0.00–0.10	0.14–0.72
	C5OH	0.01–0.11	0.12–0.30
Isovaleric acidemia	C5	0.06–0.62	52.96–60.47
Methylcrotonyl-CoA carboxylase deficiency (incl. maternal)	C5	0.06–0.62	15.52–18.38
	C5OH	0.01–0.11	0.80
Multiple carboxylase deficiency – holocarboxylase synthetase and biotinidase deficiencies	C5OH	0.06	
2-Methylbutyryl-CoA dehydrogenase deficiency	C5	0.06–0.52	1.4–2.4
2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency	C5OH	0.01–0.11	
	C5:1	0.00–0.51	
Glutaric acidemia	C5DC	3.00–0.10	0.46–1.34
Medium-chain acylCoA dehydrogenase deficiency	C8, C10, C8, C8.1		
2,4 Dienoyl-CoA reductase deficiency	C 10:2		

[a]95th percentile of the reference range. Abbreviations include DC-dicarboxylic acid.

impairment of weight gain and negative nitrogen balance. In disorders in which the organic acid is a product of amino acid metabolism, such as methylmalonic aciduria, we also measure concentrations of amino acids in plasma, and, while our patients have levels of the precursor amino acids much lower than those usually recommended as normal, we keep them above those at which weight gain stops or nitrogen balance becomes negative [4]. We maintain intake between such floor levels and a ceiling at which the plateau is exceeded and metabolite levels rise.

Quantification of organic acid analysis is essential for management; it may also be important in diagnosis. For instance, the presence of hydroxyisovalerate, hydroxypropionate and methylcitrate may suggest a diagnosis of multiple carboxylase deficiency, but these compounds are also found in propionic acidemia. The two are readily distinguished by quantification. In multiple carboxylase deficiency, the amounts of hydroxyisovalerate are large and those of the other compounds small, while in propionic acidemia, the reverse is found. Misdiagnosis of propronic acidemia as multiple carboxylase deficiency has been catastrophic.

Other methodology has been applied to the detection of organic acids. Nuclear magnetic resonance (NMR) spectrometry has become available for these purposes as the resolution of the machines has improved considerably [5]. The ability to test urine or other biological fluids without complex sample preparation raises the possibility of much more rapid diagnosis. Wider applicability should reduce the cost of diagnostic procedures. The application of MS/MS [2] to the detection of organic acidemias is of particular benefit in emergencies, for it shortens the time required for diagnosis.

Untargeted metabolomics revealed that elevated tyrosine resulting from treatment of alkaptonuria with nitisinone led to proportional elevations in alternative metabolic products, N-acetyltyrosine and γ-glutamyltyrosine [6]. This study revealed elevated levels of alterations in the pathway of metabolism of tryptophan [7]. It was clear that 4-hydroxyphenylpyruvate (lactate) correlated highly with levels of indolepyruvate (lactate). Tyrosine itself was not a direct cause of indole elevation, because patients with tyrosinemia type 2 in whom tyrosine transaminase is deficient do not have elevated indoles. Indolecarboxaldehyde, also elevated, was found to result from metabolism by intestinal bacteria.

Organic acid analysis and the occurrence of unique metabolites has led to highly accurate, rapid methods of prenatal diagnosis by GCMS of the amniotic fluid, especially with selected ion monitoring and stable isotope dilution internal standards [8]. Most experience is with analysis for methylcitrate and methylmalonic acids in the prenatal diagnosis of propionic acidemia and methylmalonic acidemia. Methodology is also available for the prenatal diagnosis of orotic aciduria [9], hepatorenal tyrosinemia [10], holocarboxylase synthetase deficiency [11], galactosemia [12], mevalonic acidemia [13], glutarylCoA dehydrogenase deficiency [14] and 4-hydroxybutyric aciduria [15].

Table 1.3 Some organic acids found in the urine in the absence of inherited metabolic disease

Compound	Situation	Inborn error in which found
Adipic acid	Gelatin; Fasting ketosis	Disorders of fatty acid oxidation
Benzoate	Bacterial metabolism, benzoate Rx, food additive, ethylene glycol	
Furane derivatives: dicarboxylate	Heated sugars; uremia	
Furoylglycine; 5-hydroxymethyl-2-furoate; furoylglycine		
Glutaric acid	Intestinal bacterial metabolism	Glutaric aciduria I and II
Glycerol	Contaminant (suppository), uremia	Glycerol kinase deficiency, fructose-6-phosphate deficiency
Glycolic acid	Ethylene glycol poisoning	Hyperoxaluria type I; 4-Hydroxybutyric aciduria
3-Hydroxyadipic acid	Fasting	LCHAD deficiency
5-Hydroxyhexanoic acid	MCT ingestion; ketosis; valproate	MCAD deficiency; multiple acylCoA dehydrogenases VLCAD, LCHAD deficiency
2-Hydroxyisocaproate	Short bowel syndrome (D-form)	Maple syrup urine Disease (MSUD); E_3 deficiency
3-Hydroxyisovalerate	Ketosis: Valproic acid	Multiple carboxylase deficiency; isovaleric acidemia; lactic acidemia, 3-methylcrotonylCoA carboxylase deficiency
4-Hydroxyphenylacetate, or-pyruvate	Intestinal bacteria	Tyrosinemia; hawkinsinuria
2-Hydroxyphenylacetic	Uremia	PKU; BH4 deficiency
2-Ketoglutaric acid	Urinary tract infection; infancy	2-KetoglutarylCoA dehydrogenase deficiency
3-Methylglutaconic acid	Uremia, pregnancy	Methylglutaconic aciduria, carbamylphosphate synthetase deficiency; propionic acidemia, β-ketothiolase deficiency
Methylmalonic acid	B12 deficiency; intestinal bacteria	Methylmalonic acidemia; transcobalamin II deficiency
N-Acetyltyrosine	Parenteral solutions	Tyrosinemia
Orotic acid	Allopurinol Rx, azauridine, folate malabsorption	Urea cycle defects, purine nucleoside Phosphorylase deficiency
Oxalic acid	Intestinal malabsorption; idiopathic; pyridoxine deficiency; rhubarb, spinach and other vegetables; ethylene glycol; ascorbic acid; methoxyflurane	Hyperoxalurias
5-Oxyproline (pyroglutamic acid)	Nonenzymatic conversion simples vigabatrin; abnormal glycine metabolism; iron oxoprolinate	Pyroglutamic aciduria; from glutamine in stored cystinosis
Palmitate	Soap; Jamaican, vomitus, sickness	
Phenylacetate; phenylacetylglutamine: phenyllactate; phenylpyruvate of urea cycle defects with phenylacetate or phenylbutyrate	Intestinal bacteria; treatment	Phenylketonuria (PKU)
Pivalate	Pivampicillin; pivmecillin	
Vanillactic acid	Bananas; neuroblastoma; carbidopa	L-Amino acid decarboxylase deficiency

Adapted from Vreken *et al.* [2]; Matern [3]; Kumps *et al.* [18].

Analysis of the organic acids of the urine may detect the presence of a disorder of neurotransmitter function, although the diagnosis is usually made by analysis of neurotransmitters or their products in cerebrospinal fluid (CSF) [16]. A patient with neonatal hypoglycemia and metabolic acidosis developed dystonia, oculogyric crises and hypothermia at eight months. He was found, on organic acid analysis of the urine, to have increased levels of vanillactic acid neonatally and later vanillpyruvic acid and acetylvanillalanine. Levels of these compounds in CSF were very high, while those of 5-hydroxyindolacetic acid and homovanillic acid were low. Enzyme assay revealed nearly undetectable aromatic L-amino acid decarboxylase activity [17].

Organic acid analysis is often confounded by the presence of compounds arising from intestinal bacterial metabolites, pharmacologic agents, nutritional supplements or nutritional deficiency. A compendium of metabolites found on organic acid analysis in inborn errors of metabolism and in other situations has been published by Kumps *et al.* [18]. Some of the common confounding metabolites are shown in Table 1.3. Organic acid analysis is commonly ordered on patients during illness, and many illnesses are accompanied by ketosis with its elevated excretion of acetoacetate and 3-hydroxybutyrate. Accompanying ketosis are increases in the excretion of 3-hydroxyisovalerate, 3-hydroxyisobutyrate and dicarboxylic acids including long-chain 3-hydroxy compounds. In this way, the pattern may be mistaken for long-chain 3-hydroxyacylCoA dehydrogenase (LCHAD) deficiency (Chapter 41), but of course in LCHAD deficiency ketonuria is inappropriately low. This distinction also rules out other disorders of fatty acid oxidation suggested by the dicarboxylic aciduria. In disorders of fatty acid oxidation, ketonuria may be present, but the ratio of urinary adipic to 3-hydroxybutyric acid is >0.5 [19]. Lactic acidemia and lactic aciduria may also be confusing because of associated increase not only in pyruvic acid, but also the branched chain keto and hydroxy acids, as found in defects of the E3 subunit of the pyruvate dehydrogenase complex.

Bacterial metabolism in the intestine is another confounding variable, which becomes particularly prominent in malabsorptive syndromes. Among the compounds found in the urine are lactic acid; this is D-lactic acid, but the chromatogram does not distinguish the D from the L forms. Specific enzymatic or other distinction must be made or the patient could be treated with oral neomycin or metronidazole and the urine reassayed. Other compounds resulting from intestinal bacteria are propionate metabolites, including methylmalonate, and aromatic compounds such as p-hydroxyphenylacetate, p-hydroxyphenyllactate, phenylacetylglutamine, phenylpropionylglycine, benzoate and hippurate. Glutaric aciduria may also result from intestinal bacterial metabolism. Bacterial urinary tract infection also produces D-lactic aciduria; increased excretion of 2-oxoglutarate is characteristic; succinate and 3-hydroxypropionate may also be increased.

The administration of valproic acid yields a number of its metabolites, which may cause confusion, but their recognition permits understanding of the secondary effects the drug has on many areas of metabolism. Organic acids found in patients receiving the drug include 3-hydroxyisovalerate, 5-hydroxyhexanoate, p-hydroxyphenylpyruvate, hexanoylglycine, tiglylglycine, isovalerylglycine and a variety of dicarboxylic acids.

Dicarboxylic aciduria is also a prominent result of the intake of medium-chain triglyceride which is found increasingly in infant formulas. 5-Hydroxyhexanoate may serve as a clue, but other medium-chain dicarboxylic acids, adipic, suberic and sebacic are found. Large quantities of adipic acid are found in the urine of children eating gelatin.

Among newly discovered organic acidemias, D-hydroxyglutaric aciduria (Chapter 11) has now been found to be caused by abnormalities in the genes for 3 different enzymes, D-2-hydroxyglutaric dehydrogenase, isocitrate dehydrogenase (IDH2) and the mitochondrial citrate carrier CICC (SLC25A1) [20]. CIC facilitates the efflux from mitochondria of intermediates citrate and isocitrate of the Krebs cycle in exchange for malate from the cytosol.

ASF_3 mutation which leads to acylCoA synthetase deficiency (Chapter 5) has been found to present with episodic ketoacidosis, methylmalonic acidemia and malonic acidemia.

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2

Propionic acidemia

MAJOR PHENOTYPIC EXPRESSION

Recurrent episodes of ketosis, acidosis and dehydration, progressive to coma; neutropenia; thrombocytopenia; osteoporosis; hyperglycinemia; propionic acidemia; methylcitraturia; and deficiency of propionyl CoA carboxylase.

INTRODUCTION

A patient with propionic acidemia was reported in 1961 [1] as having hyperglycinemia, a disorder of amino acid metabolism. Its most prominent feature was recurrent attacks of ketoacidosis. Analysis of the amino acids of blood and urine revealed very large quantities of glycine. Attacks were related to the intake of protein, and it was shown that ketonuria resulted regularly from the administration not of glycine, but of branched-chain amino acids and threonine and methionine [1, 2]. The discovery of a group of patients with hyperglycinemia who had none of these characteristics led us to coin the term "nonketotic hyperglycinemia" (Chapter 22) to distinguish them from the original group that we called "ketotic hyperglycinemia". The discovery of methylmalonic acidemia in a group of patients who displayed the ketotic hyperglycinemia syndrome [3–5] led initially to the thought that all these patients had methylmalonic acidemia. However, study of our initial patient and his sister, by Rosenberg and colleagues [6], indicated that neither excreted methylmalonic acid, and that they had propionic acidemia as a result of defective activity of propionyl CoA carboxylase (Figure 2.1). This enzyme is the first step in the pathway of propionate metabolism in which propionyl CoA, the product of the metabolism of isoleucine, valine, threonine, and methionine is converted to methylmalonyl CoA then to succinyl CoA and oxidation in the citric acid cycle.

The enzyme is composed of two subunits, α and β in an $\alpha_4\beta_4$ heteropolymeric complex. The apoenzyme is activated by the covalent binding of biotin to the amino group of lysine of the subunit. cDNA clones have been isolated for the α and β genes [7]. The α gene is on chromosome 13 and the β gene on chromosome 3. The nature of a number of mutations has been defined [8–11].

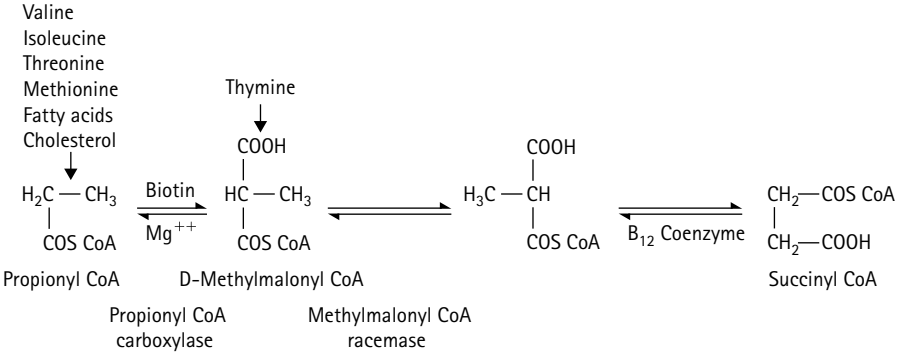

Figure 2.1 Metabolism of propionic acid. Propionyl CoA carboxylase is the site of the defect in propionic acidemia.

CLINICAL ABNORMALITIES

Patients with propionic acidemia usually present first with life-threatening illness very early in life (Figure 2.2). Many patients have died in the course of one of these episodes of illness. Patients with metabolic disease, which presents this way in the neonatal period, may appear to have sepsis, ventricular hemorrhage or some other catastrophic process. It is likely that most patients die undiagnosed. A typical episode is heralded by ketonuria. The initial symptom is often vomiting, and some patients have had such impressive vomiting that they have been operated on with a diagnosis of pyloric stenosis [1, 11, 12]. Massive ketosis leads to acidosis and dehydration. Lethargy is progressive to coma. Unless the patient is treated vigorously with intubation and assisted ventilation, as well as very large quantities of fluid and electrolytes, shock intervenes and the outcome is death [13]. Presentation of a gravely-ill infant can be with hypothermia. In an experience with 30 patients [14], 90 percent presented with severe acidosis.

Ketotic episodes are recurrent. They often follow infection, and, furthermore, at least in infancy, the untreated patient appears to be unusually susceptible to infection. We have seen a number of patients in whom septicemia, especially with klebsiella, has been documented (Figure 2.2). Initial presentations in some patients may mimic an immunodeficiency disease. Episodes are also related to diet; patients are intolerant of the usual dietary quantities of protein. A recurrent pattern of illness follows admission to hospital, correction of acidosis, and a period of no protein intake, after which the patient appears well. Feeding of the usual quantity of protein is reinitiated and the patient sent home, where ketosis recurs as soon as toxic quantities of intermediates have reaccumulated.

Clinical chemistry reveals dramatic acidosis during the acute episodes. Arterial pH values as low as 6.9 may be seen, and the serum bicarbonate may be as low as 5 mEq/L or less. There is an anion gap. To some extent, this reflects the propionic acidemia, and there is lactic acid accumulation as well, but most of the acidosis results from accumulation of 3-hydroxybutyrate and acetoacetate. Symptomatic hypoglycemia may occur.

Some neonatal presentations of propionic acidemia are with hyperammonemia and coma, suggesting a disorder of the urea cycle; ammonia levels well over 1000 μM are not unusual. Most patients have typical ketoacidosis at this time, but some do not, making the differential diagnosis difficult. The presence of neutropenia and thrombocytopenia may provide a clue to the presence of an organic academia, and some infants have pancytopenia (Figure 2.3). Amino acid analysis reveals the typical elevation of glycine, as well as of glutamine in the hyperammonemic patient. Interestingly, episodes of recurrent illness after infancy almost never lead to clinically significant elevation of ammonia.

Infants with propionic acidemia are impressively hypotonic, and this may lead to delay in achieving developmental milestones even in patients that are ultimately developmentally normal. Our initial patient had impaired mental development and microcephaly [15]. Many of these patients have impaired mental development [16, 17]. Despite mild to moderate cognitive impairment, focal neurologic abnormalities appear to be rare [17]. Atrophy has been observed on magnetic resonance imaging (MRI) of the brain [17]. Seizures and abnormalities of the electroencephalogram (EEG) have been observed. Of 11 early onset patients reported by Surtees *et al.* [18], all died; ages at death ranged from 6 days to 8 years. No patient had an IQ greater than 60. Among nine patients with later onset (6 weeks to 24 months) two died, and all had IQs greater than 60.

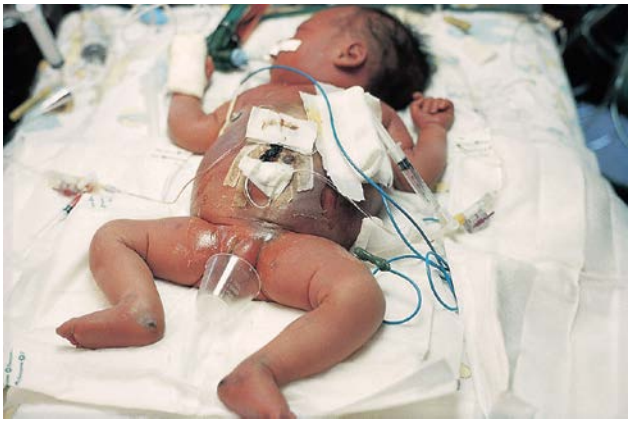

Figure 2.2 C: An infant with overwhelming illness.

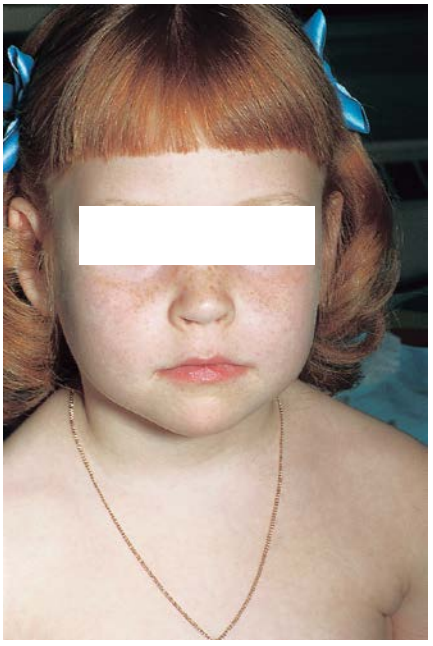

Figure 2.3 LS: A four-year-old girl with propionic acidemia. Despite a neonatal presentation, at an evaluation at 18 years of age she was normal cognitively. However, she died in a typical ketoacidotic episode at 31 years.

We have thought that the cognitive and neurologic sequelae in this disease were more likely consequences of repeated overwhelming illness early in life, with attendant shock and diminished perfusion of the brain, than of the metabolic abnormality directly. This was consistent with experience with patients treated promptly and effectively who went on to develop normally into their teens (Figure 2.3) and with a few adult patients (Figure 2.4). The sister of the first patient was diagnosed prior to the development of any symptoms, and protein restriction was initiated immediately and carried out effectively [19]. Despite the occurrence of ketoacidosis with infection, she developed normally and was intellectually fine, at most recent report, at over 30 years of age. Some of the patients of Surtees *et al*. [18] were of normal intelligence. One was diagnosed presymptomatically because his brother, whose onset was at 13 months, had the disease, and the presymptomatically diagnosed brother was alive and of normal intelligence and neurologic examination at one year of age. Hyperammonemia over 200 μM was found in four of the early onset group and only one of the late onset group (the brother of the presymptomatically diagnosed patient).

Nevertheless, a small population of patients with propionic acidemia has had a virtually exclusively neurologic presentation, sometimes without much ketoacidosis. Hypertonia may follow hypotonia or hypotonia may persist (Figure 2.5). Choreoathetosis and dystonic posturing have been observed. Deep tendon reflexes are exaggerated and the Babinski response may be present.

In two patients with an exclusively neurologic presentation [20], the life-threatening episodes of ketoacidosis that usually serve as alerting signals were absent. In addition, hyperammonemia was prominent in late infancy in one and as late as 15 years in the other. Hypotonia, spastic quadriparesis and choreoathetosis were major manifestations. One patient displayed self-injurious behavior with mutilation of his lower lip (Figure 2.6). Choreoathetosis, pyramidal tract signs and dystonia have also been reported in other patients [19], including an infant who did not have ketoacidosis or hyperammonemia [21].

An infant who presented with a pure hyperammonemia picture without ketoacidosis is shown in Figure 2.7. MRI of the brain revealed extensive atrophy (Figure 2.8). An unusual patient [22] was diagnosed at 31 years of age after admission to a psychiatric hospital where he was admitted for bizarre behavior and studied further because of involuntary movements. We have observed MRI evidence of hypodense myelin, along with areas of increased signal in the basal ganglia [20]. We have also encountered a metabolic stroke in an eight-year-old patient with propionic acidemia in which there was virtually complete infarction of the basal ganglia followed by death [23, 24]. We have been informed about a similar patient who did not die, but remained in a vegetative state. A 15-year-old diagnosed neonatally suddenly developed a stroke of the basal ganglia from which he ultimately recovered [25]. Assessment of cerebral vessels

Figure 2.4 KZ: A 22-year-old Costa Rican girl with propionic acidemia. Two previous siblings had died with identical symptoms to those that she presented with in the early months of life. One sibling was operated on for pyloric stenosis, but at surgery, the pylorus was deemed normal. The patient's presentation included multiple episodes of metabolic ketoacidosis requiring admission to hospital following diagnosis at two years. There were no further admissions for acidotic imbalance. Since this picture was taken, we have been informed that she died.

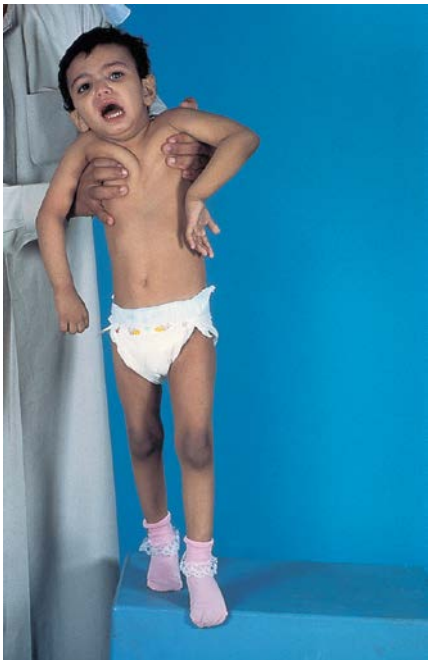

Figure 2.5 A four-year-old Saudi patient with propionic acidemia who was still impressively hypotonic.

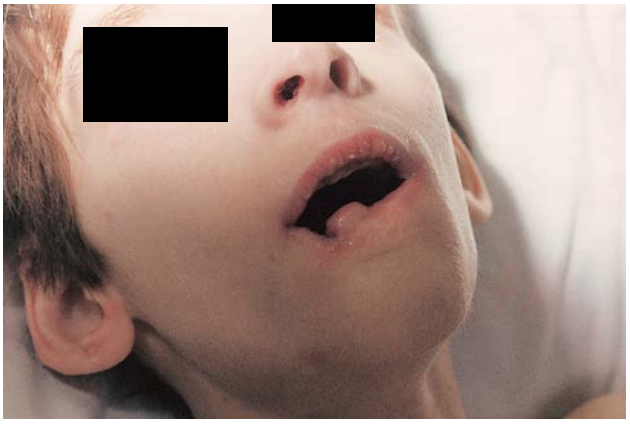

Figure 2.6 A 20-year-old man with propionic acidemia who presented with severe impairment of cognitive function, spastic quadriparesis and a mutilated lip that led to his referral as a patient with Lesch-Nyhan disease. HPRT assay was normal and metabolic exploration led to the diagnosis.

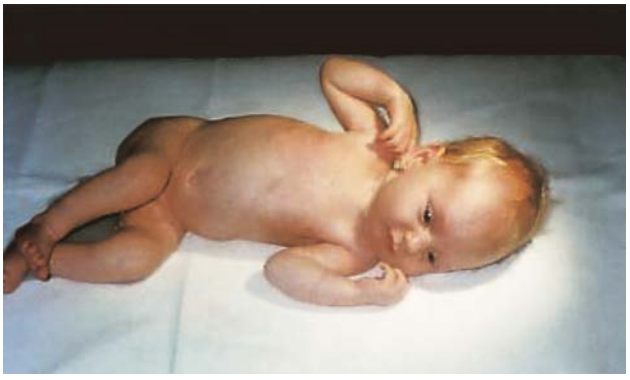

Figure 2.7 An infant with propionic acidemia who presented acutely at 20 days of age in coma with a blood ammonia of 450 μmol/L and no ketoacidosis. A brother had died at 40 days after an identical clinical presentation.

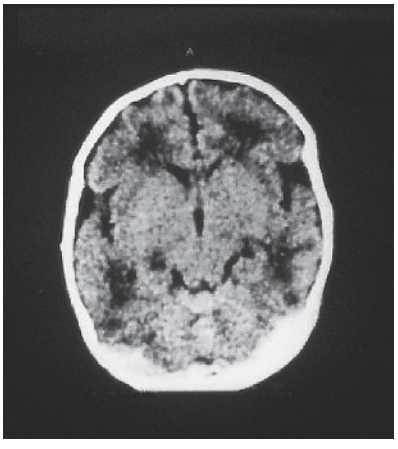

Figure 2.8 MRI of the brain of the infant in Figure 2.7, illustrating extensive cerebral atrophy. (Illustration and Figure 2.8 were kindly provided by Dr I Baric of the University Hospital Center, Zagreb, Croatia.)

showed no abnormality. Treatment with L-DOPA appeared to be beneficial.

Patients with propionic acidemia also regularly have neutropenia at the time of diagnosis. It is responsive to treatment of propionic acidemia (*vide infra*) and may reappear with recurrent metabolic imbalance. Transient thrombocytopenia is seen in infancy. Rarely, there may be anemia [26]. These hematological effects mirror the effects of propionyl CoA on marrow cell development, and they respond to metabolic control. Chronic moniliasis occurs in this syndrome, as well as in methylmalonic acidemia. This problem reflects the effect of propionyl CoA on T-cell number and function and particularly their response to candida [27, 28]. In the series of Lehnert *et al.* [14] skin lesions were found in 53 percent; in addition to candida, they encountered staphylococcal scalded skin syndrome, alopecia in two patients, and flaky or lamellar desquamation around the mouth or perineum that was called "dermatitis acidemica". In our experience, most of these noncandidal skin problems could be attributed to deficiency of protein or a specific amino acid often in a patient under excellent control who suddenly developed infection.

Osteoporosis is a regular concomitant of this disease and may be so severe that pathological fractures occur [2]. Diminished bone density may be documented even in patients maintained in excellent metabolic control.

Acute and recurrent pancreatitis has been observed as a complication of this disease [23], as well as other organic acidemias. In these patients, vomiting and abdominal pains are associated with elevated levels of amylase and lipase.

For reasons that are not clear, patients have been observed who have no symptoms of disease, at least to the time of the report at teenage, despite documentation of virtually no enzyme activity and ascertainment through symptomatic siblings [29]. We have not encountered such patients, nor have those reporting experience with large numbers of patients [14, 30].

Infants with propionic acidemia tend to resemble each other and those with methylmalonic acidemia (Figure 2.9). Characteristic facial features are: frontal bossing; widened depressed nasal bridge, and an appearance of wide-set eyes; epicanthal folds, and a long filtrum with upward curvature of the lips. In addition, the nipples may be hypoplastic or inverted (Figure 2.10).

Neuropathologic findings [31, 32] in patients dying in the neonatal period have been those of spongy degeneration of the white matter. In patients dying later, abnormalities in the basal ganglia were prominent [23, 31]. These included gross shrinkage and marbling, as well as microscopic neuronal loss and gliosis.

Among late complications of inherited metabolic diseases, cardiomyopathy is emerging as a major complication of propionic acidemia [32–35]. Clearly, this may be fatal. Fatal hypertrophic cardiomyopathy was found at autopsy in a patient despite therapy with carnitine and absence of an acute episode of decompensation [34].

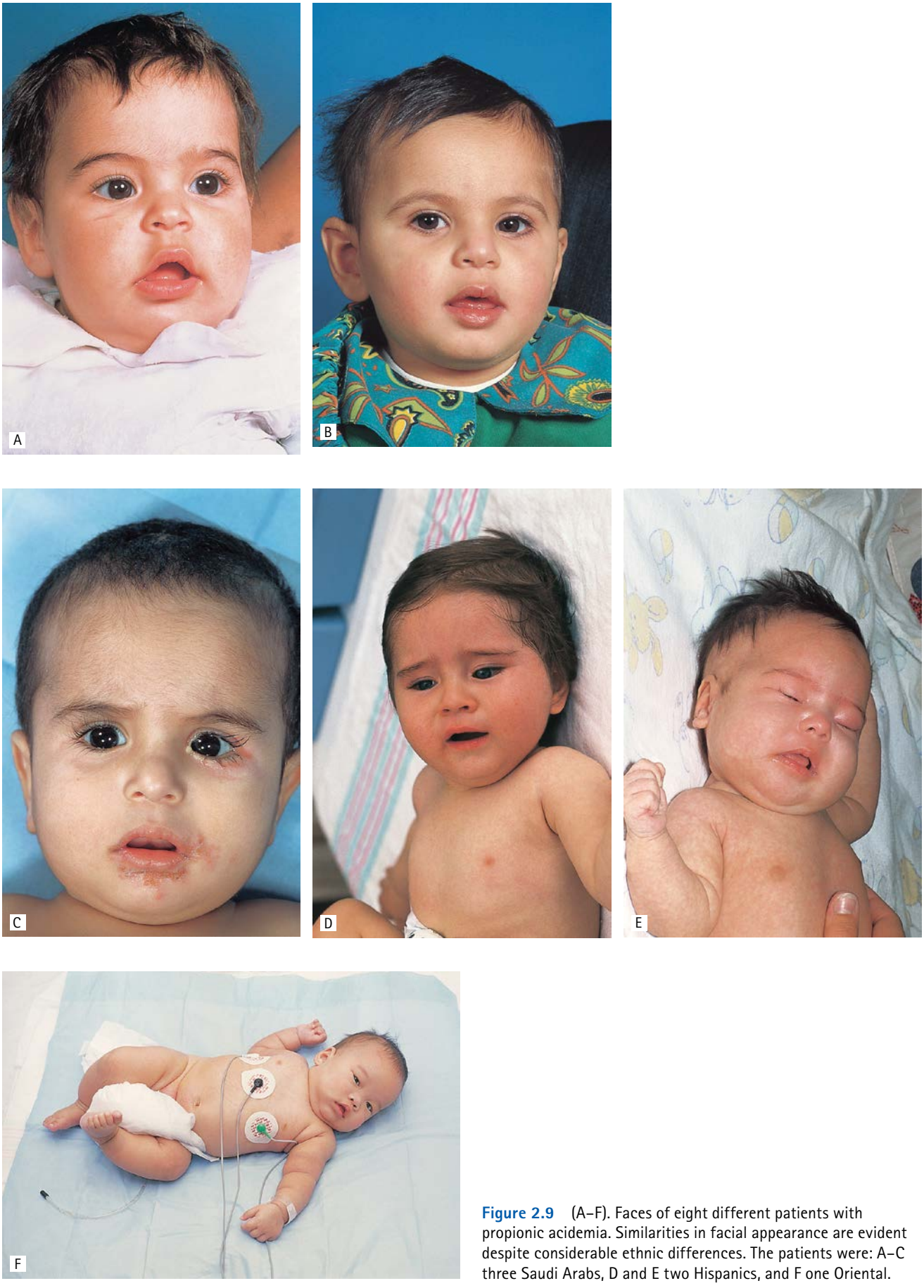

Figure 2.9 (A–F). Faces of eight different patients with propionic acidemia. Similarities in facial appearance are evident despite considerable ethnic differences. The patients were: A–C three Saudi Arabs, D and E two Hispanics, and F one Oriental.

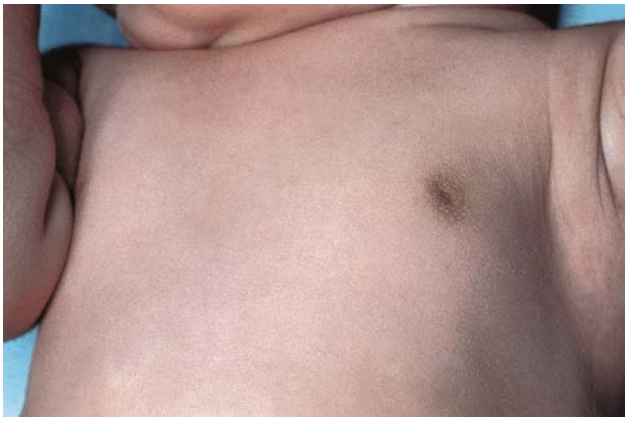

Figure 2.10 Inverted nipples in a patient with propionic acidemia.

Concentrations of carnitine in cardiac muscle were found to be low.

GENETICS AND PATHOGENESIS

Propionic acidemia is inherited as an autosomal recessive trait. The enzymatic site of the defect is propionyl CoA carboxylase [36, 37]. Activity in extracts of leukocytes and fibroblasts is very low, usually less than 5 percent of control (Table 2.1). Studies with somatic cell hybrids have provided evidence of two complementation groups, PccA and PccBC, which correspond to abnormalities in the α and β subunits, respectively [38–43]. The BC group contains two subgroups, B and C, in which intragroup complementation is thought to be interallelic. Patients in the A subgroup have mutations in the A gene for the α chain, and those in the BC groups have mutations in the B gene for the β chain. Residual activity of propionyl CoA carboxylase correlates poorly with severity of disease or outcome [14].

Heterozygosity is not reliably determined by assay of the enzyme in cultured fibroblasts. A positive indicates heterozygosity, but a negative may not be consistent with its absence. Heterozygotes for the PccA group display approximately 50 percent of control activity of the enzyme, but those of the PccBC group are not distinguishable from normal [38].

Table 2.1 Propionyl-CoA carboxylase activity (picomol of 14C bicarbonate fixed/mg protein/min) in patients with propionic acidemia

	Normal	Patients
Lymphocytes		
Mean ± 1 SD	232 ± 87	10 ± 9
(Range)	(160–447)	(0–36)
n	45	23
Fibroblasts		
Mean ± 1 SE	294 ± 94	15 ± 17
(Range)	(128–537)	(0–51)
n	36	10

Immunochemical assay of the PccA group has revealed many with little or no α chain of the enzyme [39] and other studies indicated an absence of α chain mRNA; these cells lack the subunit which is thought to have been degraded while the β chain mRNA was present [43]. This is consistent with the expression of 50 percent of activity in heterozygotes. Cells of the BC groups may contain immunoprecipitable subunits but lack β subunits [44, 45]. The normal activity in BC heterozygotes is thought to result from a five-fold greater synthesis of β subunits than a unit. The amount of residual carboxylase activity measured in patients is thought to reflect the activity of other carboxylases on the substrate.

The cDNAs for the α [46] and β [47, 48] subunits have been cloned, and the genes have been mapped, respectively, to chromosomes 13q32 [49] and 3q13.3-22 [50]. Both genes are very large. The tetrapeptide sequence, Ala-Met-Lys-Met in the amino acid sequence of the chain deduced from the gene [7] appears to be a universal feature of the binding site of all carboxylases.

A number of mutations has been defined at the level of the DNA; a recent counting was 27 mutations in PCCA and over 30 in PCCB [9, 11, 48, 51–53]. Among mutations in the A gene, nonsense and splicing mutations, which cause exon skipping and deletions, have led commonly to an absence of mRNA [54]. In a patient with a mild form of disease frameshift mutation 440delC was missed on RT-PCR because it was not expressed in the mRNA [55]. Among point mutations in this gene, abolition of biotin binding was common [56, 57]. Among mutations in the B gene, there have been a number of missense mutations, such as C to T change, that changed an arginine at residue 410 of the β subunit to a tryptophan [52], which was common in Japanese patients; and an insertion/deletion (1218del14ins12) with a frameshift and a stop codon, that has been common in Caucasian cell lines studied [9, 51]. A frequent mutation in Spanish patients was 1170insT [58]. However, the 1218del14ins12 was found in 31 percent of Spanish and 44 percent of Latin American alleles [8, 59].

Prenatal diagnosis [59–63] has been accomplished by measurement of activity of propionyl CoA carboxylase in cultured amniotic fluid cells [59] or chorionic villus cells [60], or fixation of ^{14}C propionate in amniocytes [61]. It is more rapidly accomplished by the direct gas chromatography-mass spectrometry (GCMS) assay of methylcitric acid in amniotic fluid [62], a method which obviates the error always implicit in cell culture approaches that those cells ultimately analyzed are maternal, not fetal [63]. It has also been accomplished by measurement of propionylcarnitine in amniotic fluid. In those families in which the mutation is known, it may be made by assay of the DNA, ideally with oligonucleotide probes.

There are a number of biochemical consequences of the defective activity of propionyl CoA carboxylase, many of which have direct relevance to the pathogenesis of the clinical manifestations of the disease. The immediately apparent consequence (see Figure 2.1) is the inability to catabolize four essential amino acids: isoleucine, valine, threonine, and methionine. These amino acids are responsible for the toxicity of protein ingested in amounts greater than required for growth, and they were shown in the initial studies [1, 2] to induce ketonuria when administered individually.

Patients with propionic acidemia have elevated concentrations of glycine in the blood and urine. This was the first of the biochemical abnormalities to be recognized [1]. It occurs along with abnormal ketogenesis, also in methylmalonic acidemia (Chapter 3), in isovaleric acidemia (Chapter 7), and in 3-oxothiolase deficiency (Chapter 13).

The mechanism of hyperglycinemia appears to be an inhibition by propionyl CoA of the synthesis of the glycine cleaving enzyme leading to defective oxidation of glycine. The hyperglycinemia of propionic acidemia is usually readily differentiated from nonketotic hyperglycinemia by the occurrence of episodes of ketosis. However, we have observed overwhelming illness without ketosis in a patient with propionic acidemia [64]. It is for this reason that all hyperglycinemic infants should be assessed for a possible diagnosis of propionic acidemia before a diagnosis of nonketotic hyperglycinemia is made.

When propionyl CoA accumulates, other metabolic products are found in the blood and urine. The predominant compound is 3-hydroxypropionic acid; others include tiglic acid, tiglyglycine, butanone, and propionylglycine. In addition, the unusual metabolite methylcitrate is formed by condensation of propionyl CoA and oxaloacetic acid [65]. This compound is an end product of metabolism and is very stable, resistant to conditions of shipment and bacterial contamination. In our hands, it is the most reliable chemical indicator of the presence of this disorder. It is useful in prenatal diagnosis, as well as the initial diagnosis. Odd chain fatty acids may accumulate in body lipids as a consequence of synthesis from propionyl CoA. They may be demonstrated and quantified in erythrocytes [66]. 3-Ureidopropionate is found in the urine [67], a consequence of propionate inhibition of ureidopropionase. The manifestations of patients with inherited deficiency of this enzyme of pyrimidine metabolism are reminiscent of those of propionic acidemic patients with changes in the basal ganglia, and there is *in vitro* evidence that ureidopropionate is neurotoxic [68]. 2-Methyl-3-oxovaleric acid, a product of self-condensation of two molecules of propionyl CoA, has been a useful metabolite for Lehnert and colleagues [14] for the diagnosis of propionic acidemia. Its reduction yields 3-hydroxy 2-methylvaleric acid. Hyperlysinemia or hyperlysinuria encountered in propionic acidemia [14] appears to reflect study during hyperammonemia, during which lysine accumulates.

Abnormal ketogenesis is a major cause of morbidity and mortality in this disease. It could result from a variety of mechanisms. Propionic acid is an inhibitor of mitochondrial oxidation of succinic and 2-ketoglutaric acid, and propionyl CoA is an inhibitor of succinate: CoA ligase, and malate dehydrogenase [69]. Carnitine prevents this, consistent with its role in therapy. Carnitine is depleted in these patients, because it forms the propionylcarnitine ester, which is excreted in the urine. Analysis for propionylcarnitine has also been used for diagnosis, and has been effectively explored in prenatal diagnosis [70]. The accumulation of propionyl CoA, and its condensation with oxalacetate to form methylcitrate depletes oxalacetate and so acetyl CoA, deprived of substrate with which to condense to form citrate, condenses with itself to form acetoacetate. A variety of mitochondrial oxidative functions have been found to be inhibited by propionic CoA [71] here including pyruvate dehydrogenase, 2-ketoglutarate dehydrogenase and decrease in the amount and activities of the OXPHOS complexes I–IV.

The hyperammonemia observed in infants with propionic acidemia is a consequence of the inhibition of the urea cycle at the carbamylphosphate synthetase (CPS) step by propionyl CoA. This results from a competitive inhibition of N-acetylglutamate synthetase [72].

The obligatory biotin cofactor has led to the possibility that some patients with propionic acidemia are biotin responsive [73]. Nevertheless, no patient has been shown to be clinically responsive to biotin. Most patients we have tested by assessing the conversion of 13C-propionate to $^{13}CO_2$ *in vivo*, before and after biotin, have shown no evidence of response [74]. The one patient in whom there was a small response had no clinical response to a course of treatment with biotin.

The advent of programs of expanded newborn screening using tandem mass spectrometry has greatly increased the yield of patients with propionic acids treated presymptomatically. It has seemed likely that mutations that convey an attenuated clinical disease may be uncovered in this way, as has been observed in other disorders, such as medium-chain acyl CoA dehydrogenase deficiency (Chapter 38), and 3-methylcrotonyl CoA carboxylase deficiency (Chapter 5), but the incidence of propionic acidemia in newborn screening does not appear to differ from incidence encountered in patients diagnosed clinically. The diagnostic analyte in dried neonatal blood spots is C3 (propionyl) carnitine. The ratio of C3/C0 is also elevated.

TREATMENT

The cornerstone of treatment is the dietary restriction of the intake of all of those amino acids whose metabolism takes them through propionyl CoA to the amounts that are required for growth and no more. We have provided these amino acids in the form of a standard cow's milk formula whose amino acid content is known. We have made up the

rest of the calories in fat and carbohydrate [75–77]. It is not necessary to supply a mixture of the other amino acids, although this approach has regularly been employed; it is certainly indicated if an individual's amino acid becomes limiting, but it is possible to manage such a patient by supplementing just that amino acid. Treatment must be monitored from time to time with quantitative assays of the relevant metabolites in the urine.

We also assess growth in weight, nitrogen balance, and the concentrations of amino acids in blood. We aim for an intake of protein below that at which plateau levels of amino acids rise and above the levels required for positive nitrogen balance, and growth and height [77]. The quantification of urinary urea [78] is a useful adjunct to the therapy. It may be useful to monitor erythrocyte concentrations of odd chain fatty acids [79]. We teach our parents to test for ketones in the urine using ketostix. Ideally, the urine is tested daily in infancy. Thereafter, it can be done at intervals, with special attention to periods of intercurrent infection.

The addition of carnitine to the therapeutic management of infants with this disease has had a major impact on management [80–84]. Patients are all carnitine-depleted in the absence of treatment. Treatment increases the excretion of carnitine esters, which should promote detoxification. It also substantially reduces the propensity for ketogenesis as tested by fasting [84]. Concomitantly, it has seemed that our patients tolerate the catabolism of infection better, and require less frequent admission to hospital. Doses generally employed have been from 60 to 100 mg/kg, although ketogenesis is less with 200 mg/kg. Doses higher than this usually produce diarrhea, but, otherwise, toxicity has not been encountered. Much higher doses can be employed parenterally without producing diarrhea.

Experience has indicated that the anabolic properties of human growth hormone have decreased the propensity for catabolism in these patients [85]. Certainly, there is improvement in growth, lean body mass, and mineralization of bone, as well as decrease in adiposity.

The treatment of the acute episode of ketoacidosis requires vigorous attention to supportive therapy. We use very large amounts of parenteral fluid and electrolytes, along with high doses of intravenous carnitine (Table 2.2). Emergency is such that blood is obtained to determine concentrations at electrolytes and bicarbonate and an intravenous infusion is started before taking time to take a history and do a physical examination. Following a bolus of 20 mL/kg of ringer lactate or isotonic saline we usually begin with isotonic (150 mEq/L) of $NaHCO_3$. Carnitine is infused at a rate of 300 mg/kg. Electrolytes are determined at least every six hours. The serum concentration of sodium should be greater or equal 138 mmol/L. The $NaHCO_3$ content of the fluid may be reduced (to 75 MEq/L) after the serum bicarbonate has become normal, but it is continued at the same rate until the ketonuria has receded to small.

Table 2.2 Management of the acute episode of ketoacidosis (intravenous)

Water (mL/kg)	$NaHCo_2$ (mEq/L)	Glucose (%)	Carnitine (mg/kg)
200	150	5–10	300

Fasting has been demonstrated [86] to increase the excretion of urinary metabolites of propionate, presumably from the oxidation of odd chain fatty acids stored in lipid. Consistent with this, studies of sources of propionate in patients with propionic acid and methylmalonic acidemia by means of ^{13}C-propionate turnover [87] indicated about 30 percent of propionate production not accounted for, suggesting that this much might come from propionate stored in lipid. Data are not available for propionate turnover in infants and children not subjected to overnight fast, which these authors had shown to increase excretion of urinary metabolites of propionate. The avoidance of fasting is recommended in this disorder. This is also an argument for the inclusion of glucose in the infusion solution; larger amounts may be beneficial. In a conscious patient without intestinal intolerance, cornstarch or polycose by mouth or nasogastric tube may be useful.

Neonatal hyperammonemia may require treatment with intravenous sodium benzoate and/or phenylacetate (Chapter 23), or hemodialysis [88]. The management of acute attacks of hyperammonemia in propinic and methylmalonic acidemia has been expanded by the use of carbamylglutamate [89–91]. This compound is an analog of N-acetyglutamate, which stimulates the activity of carbamylphosphate synthetase. It has been documented to lower levels of ammonia in patients not responding to treatment with sodium benzoate, arginine and glucose intravenously. Best results were obtained in a patient in whom treatment of hyperammonemia was started before a specific diagnosis was achieved [91]. In patients reported, the other treatment was continued. Carbamylglutamate was given intravenously in a dose of 150 mg/kg, followed by 100 mg/kg daily.

Parenteral mixtures of amino acids, in which the concentration of isoleucine, valine, threonine, and methionine are reduced or absent, may be useful, especially in the patient with intestinal abnormalities [92]. Insulin may be a useful adjunct [93]. The efficacy of growth hormone in the acute episode has not been assessed.

Studies of propionate production before and after treatment with metronidazole in three patients with propionic acidemia and three with methylmalonic acidemia [94] indicated that a mean of 22 percent could be attributed to formation of propionate by intestinal bacteria. However, the data were quite variable. In the patient with methylmalonic acidemia with the lowest level of methylmalonate excretion in the urine, the excretion after metronidazole was little changed, and the propionate turnover changed only from 46 to 41 μmol/kg per hour, which does not seem significant. Similarly, these authors reported an average reduction of excretion of propionate metabolites of 41 percent in nine patients with disorders of propionate metabolism treated with metronidazole [86].

In our experience, results have been quite variable as measured by change in metabolite excretion following treatment with oral neomycin or metronidazole even in the same patient, suggesting that the intestine may be colonized by varying clones or groups of organisms, which do or do not make propionate. The reported data [86] are consistent with this, in that two patients with low levels of metabolites excreted had barely appreciable changes after metronidazole from 1.9 to 1.6 μmol/kg per hour total metabolites and 4.0 to 2.9 even though the percentage decreases were 16 and 29 percent. A trial of antibiotic treatment is always worthwhile, and it is especially indicated by a change in excretion in a patient whose pattern is well known, and there is no obvious cause for catabolism. We prefer to start with neomycin because it is not absorbed. We have used a dose of 50 mg/kg. Metronidazole has been used in doses of 10–20 mg/kg.

Transplantation of the liver has been employed in propionic acidemia [95, 96]. As overall results of the procedure in children have improved, the results in propionic acidemia have become more encouraging. The metabolic abnormality is not corrected, but there may be a five-fold decrease in methylcitrate excretion, and there may be a major reduction in propensity to ketoacidosis. A patient with neonatal onset disease transplanted at 7 months, experienced dramatic improvement in EEG; neurologic development and MRI of the brain were judged age appropriate at 2 years of age [95].

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3

Methylmalonic acidemia

MAJOR PHENOTYPIC EXPRESSION

Recurrent episodes of ketosis, acidosis, vomiting, and dehydration; anorexia, failure to thrive; hepatomegaly; osteoporosis; neutropenia; thrombocytopenia; hyperglycinemia; elevated concentrations of methylmalonic acid (MMA) in blood and urine; and defective activity of methylmalonyl CoA mutase.

INTRODUCTION

Methylmalonic acidemia represents a family of disorders of the metabolism of branched-chain amino acids in which the activity of methylmalonyl CoA mutase is defective (Figure 3.1). Patients with the inborn error of metabolism were first reported in 1967 by Oberholzer *et al.* [1] and by Stokke *et al.* [2]. In 1968, Rosenberg and colleagues [3] first clearly distinguished these patients from those with propionic acidemia (Chapter 2), in whom the clinical presentation is often virtually identical.

Genetic heterogeneity was evident early in the demonstration, in that some patients with methylmalonic acidemia were responsive to large doses of vitamin B_{12}, while others were not [4]. The methylmalonyl CoA mutase enzyme has a vitamin B_{12}-derived cofactor, adenosylcobalamin (AdoCbl). Patients who are B_{12}-responsive clinically have defects in the synthesis of the cofactor. Unresponsive patients have defects in the apoenzyme itself. Complementation studies have indicated the presence of distinct groups (Figure 3.1). Those with apoenzyme defects have been designated mut$^-$ or mut^0 depending on whether they have little or no residual mutase activity. Groups A and B represent defects in AdoCbl synthesis. A differential diagnosis of methylmalonic acidemia is shown in Table 3.1.

The cobalamin (Cbl) C and D represent a different type of disorder in which methylmalonic acidemia accompanies elevated concentrations of homocysteine and cystathionine in blood and urine (Chapter 4) [5]. In these groups, defective remethylation of homocysteine to methionine is the consequence of a failure to transform B_{12}

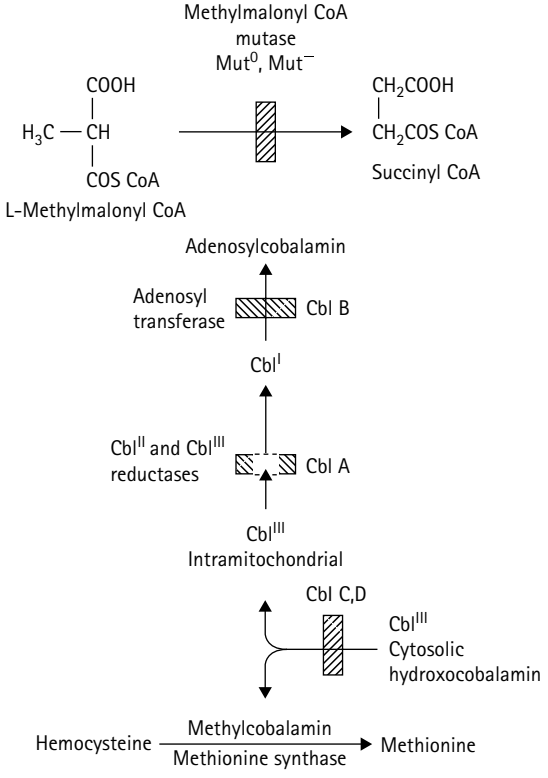

Figure 3.1 Methylmalonyl CoA mutase, the site of the defect in methylmalonic aciduria is shown above its cofactor adenosylcobalamin (AdoCbl). Cobalamin cofactor synthesis is also illustrated in the formation of AdoCbl. The sites of the defects in the various complementation groups identified are shown as Cbl A, B, C and D. Apoenzyme defects are referred to as Mut^0 or Mut$^-$.

Table 3.1 Different types of methylmalonic acidemia

Methylmalonyl CoA mutase deficiency (mut^0, mut^-)
Adenosyltransferase deficiency (MMAB) (Cbl B)
G protein Chaperon (cobalamin MMAA)
Cbl A
Homocystinuria with methylmalonic acidemia (MMA) (Cbl C, D) (Chapter 4)
Methylmalonyl CoA epimerase deficiency
B_{12} deficiency (vegan mother – breast feeding) (vegan child)
Pernicious anemia (intrinsic factor deficiency)
Methylmalonyl CoA epimerase deficiency
Transcobalamin II deficiency
B_{12} transport from lysosome defect (Cbl F)
Succinyl CoA ligase deficiency (SUCLG1, SUCLA2)

to either of the coenzymatically active derivatives, AdoCbl or methylcobalamin. Cbl F disease reflects abnormalities in the transport of cobalamin out of lysosomes, analogous to the defect that causes cystinosis. Methylmalonic aciduria is also seen in acquired deficiency of B_{12} [6], in pernicious anemia and in transcobalamin II deficiency [7]. In B_{12} deficiency and in intrinsic factor deficiency, the excretion of MMA in the urine is a more reliable index of depletion of body stores of cobalamin than the blood level of B_{12}. Methylmalonic acidemia resulting from a defect in the metholmalonyl, CoA epimerase enzyme, long suspected to exist has now been documented by mutational analysis in a homozygous patient [8, 9]. Methylmalonic aciduria of modest degree has been the clue to a metabolic diagnosis in patients with succinyl CoA ligase (synthase) deficiency which leads to a mitochondrial DNA depletion syndrome [10–13]. This complex has two subunits, α and β, coded by genes *SUCLA2* and *SUCLG1*. Mutations in both have been documented [11, 13].

All of the methylmalonic acidemias reflect defective activity of methylmalonyl CoA mutase [14]. In inherited defects of the apoenzyme and in abnormalities in coenzyme synthesis, the enzymatic mutase abnormality is evident in tissues, leukocytes, and cultured fibroblasts. The mutase gene has been cloned [15] and mapped to chromosome 6 [16]. Some 200 mutations in the mutase gene have been documented [17–19].

CLINICAL ABNORMALITIES

Patients with methylmalonic acidemia usually present first with a typical organic acidemia picture of overwhelming illness very early in life [1–3, 20–23]. A majority of the reported patients, especially those with apoenzyme defects, have died in such an episode. We believe that prior to newborn screening, many patients died with the disease unrecognized, and that the disease is more common than previously realized. A typical episode is ushered in with ketonuria and vomiting, followed by acidosis, dehydration, and lethargy, leading, in the absence of aggressive treatment, to coma and death.

Episodes of acute illness are recurrent. They may follow even minor infections. Furthermore, patients are unusually prone to infection. Episodes are also a consequence of feeding: these patients are intolerant of the usual quantities of dietary protein. More specifically, they are intolerant to the amino acids isoleucine, valine, threonine, and methionine, all of which are catabolized through the pathway of propionate and methylmalonate metabolism (see Figure 2.1). Episodic disease may follow a pattern in which the patient is admitted to hospital *in extremis*, treated vigorously with parenteral fluid and electrolytes, which leads to recovery; oral feedings are reintroduced and, following a sufficient time of ingestion of the usual dietary amounts of protein, another episode of crisis supervenes, and in one of these episodes, the patient dies.

During episodes of ketosis, acidosis may be extreme. Arterial pH values as low as 6.9 have been recorded, and the serum bicarbonate is often 5 mEq/L or less. Ketosis is massive. Hypoglycemia has been observed and has led to seizures during acute episodes. Elevated concentrations of glycine in the blood and urine may be striking, and this may be an early clue to the diagnosis. Concentrations of glycine as high as 1500 μmol/L have been observed in the plasma. However, concentrations of glycine may also be normal, even in the same patient. Hyperammonemia may complicate the initial episode in which levels may be as high as in urea cycle defects and lead to deep coma and apnea [24, 25]. With development, this propensity to hyperammonemia is lost, and acute episodes after the first year are seldom complicated by hyperammonemia [24]. The typical acute episode of disease with massive ketosis has led us to examine the urine for ketones in any acidotic infant. In addition, in management we provide ketostix to parents and instruct them to test for ketones at any sign of illness, especially infections; on the other hand, this too can be misleading. We are currently following an adult with mut^0 methylmalonic acidemia who develops acute episodes of acidosis with no ketonuria. Kussmaul breathing is still an alerting feature.

Failure to thrive may be the initial presentation in this disease and failure of linear growth may be striking (Figures 3.2, 3.3, and 3.4). Developmental failure may parallel the inability to increase weight, height, and head circumference. Anorexia is severe, and usually requires tube feeding (Figure 3.3). In addition to the fact that the ketoacidotic episode is often ushered in with vomiting, these patients vomit frequently in infancy, and this may contribute to failure to thrive.

A variety of skin lesions may be seen (Figure 3.5). Most often, this is a manifestation of moniliasis. Mucocutaneous moniliasis may also be reflected in cracking and erythema at the angles of the mouth and the eyes (Figure 3.3) [26].

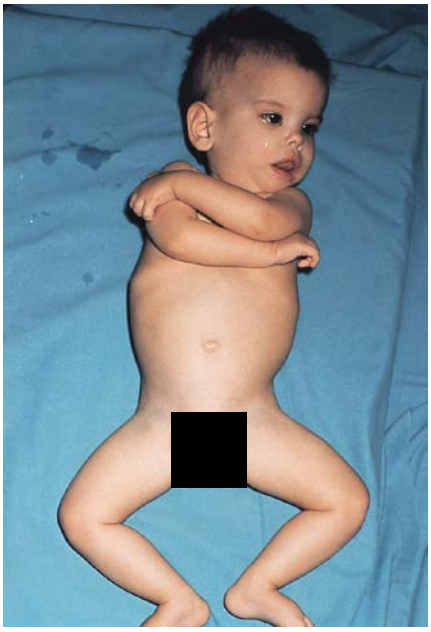

Figure 3.2 LG: A 14-month-old girl with methylmalonic acidemia. The size, that of a three-month-old infant, reflects the severe failure to thrive characteristic of this disorder. The frog-leg position illustrates the marked hypotonia.

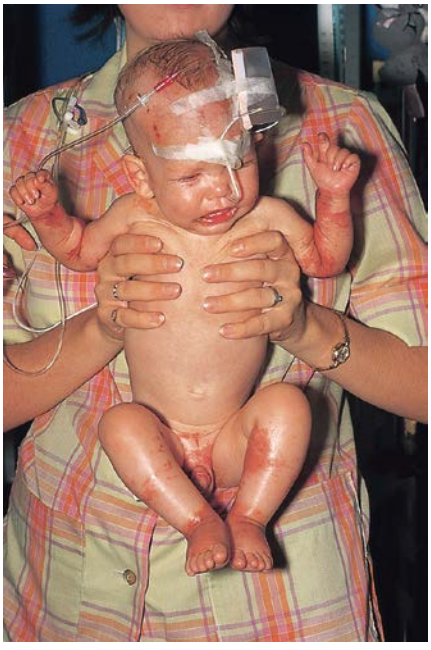

Figure 3.3 ME: A 13-month-old boy with methylmalonyl CoA mutase deficiency who also failed to gain in weight, height, or head circumference since the age of three months. The nasogastric tube is typical, indicating the extreme anorexia. The bright red erythematous lesions are the characteristic monilial infection of the infant in poor metabolic control.

Patients with methylmalonic acidemia have a striking resemblance to each other, especially in infancy (Figure 3.6). The characteristic face includes a high forehead, broad nasal bridge, epicanthal folds, a long smooth filtrum, and

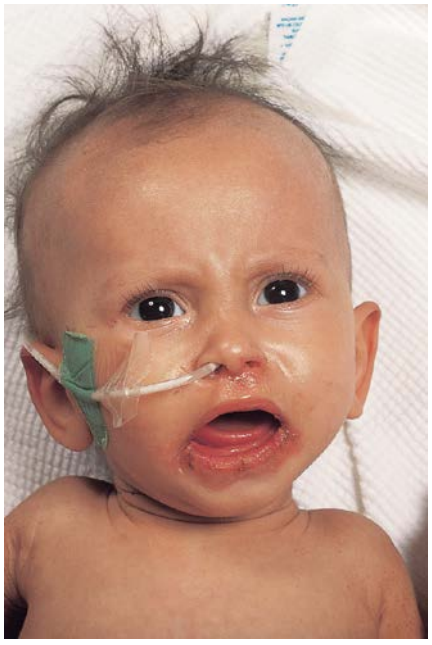

Figure 3.4 This infant with methylmalonic acidemia had also failed to thrive and was anorexic. She also had alopecia.

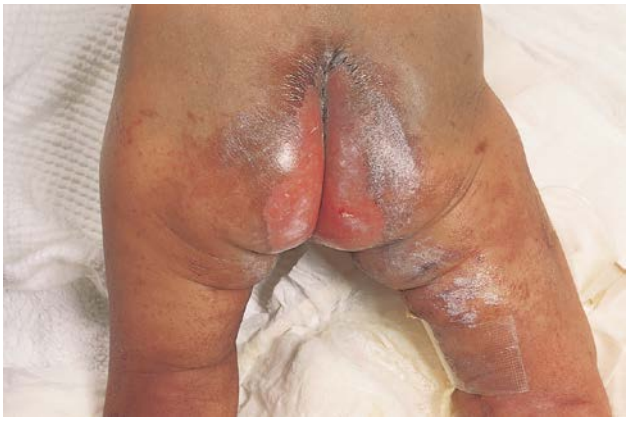

Figure 3.5 The same infant had a florid perineal dermatitis.

a triangular mouth. A few have had other minor anomalies [23]. A recent patient of ours had inverted nipples. One patient had multiple defects at birth, including cardiac septal defects, hydronephrosis, and an appearance of Sotos syndrome [27].

Neurologic manifestations of methylmalonic acidemia are varied. In infancy and childhood, these features appear to be more consequences of the physiology of the acute episode of shock and diminished cerebral perfusion or hypoglycemia, and especially hyperammonemia with or without cerebral edema, than the metabolic abnormality itself [28]. Developmental impairment is evident in most patients in infancy, but in some this may be more apparent than real; evidence of severe chronic disease and extreme hypotonia, both of which interfere with motor development. Catch up has been observed in patients successfully treated, and the IQ may be normal [23, 28].

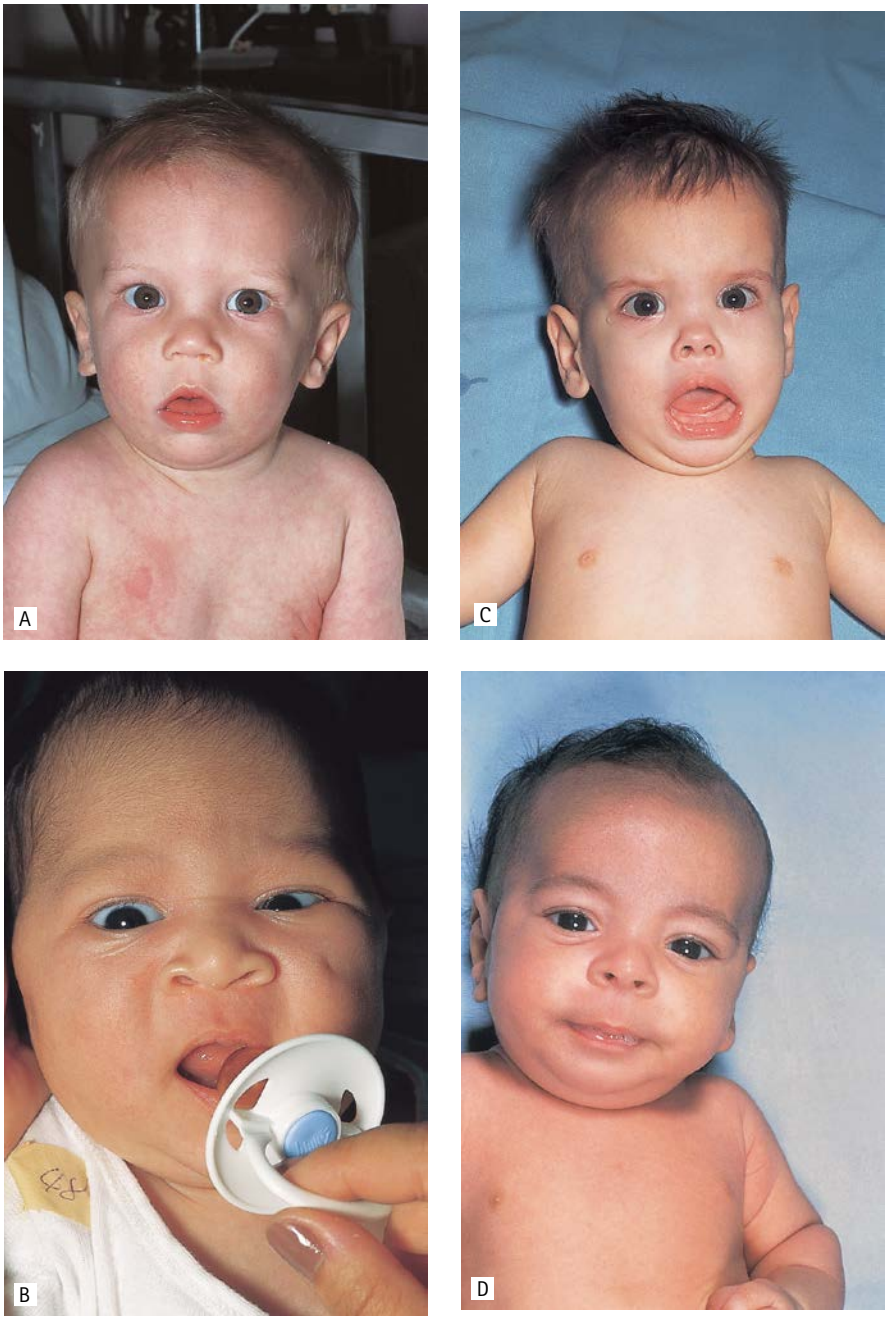

Figure 3.6 (A, B, C, D) Composite picture of four patients with methylmalonic acidemia highlighting the similarity of the facial features. A high forehead, broad nasal bridge and wide-appearing eyes with epicanthal folds and a long smooth filtrum were characteristic. In some, the nose was upturned and in some, the mouth was triangular. The patient in panel (C) had had a preauricular skin tag which was removed.

The impairment and disability phenotype of 33 patients with isolated methylmalonic acidemia has been set out [29]. Seventeen had lesions in the globus pallidus on magnetic resonance imaging (MRI). Neurologic findings, including ataxia, dystonia, dyskinesia, dysarthria, chorea, clonus, extrapyramidal signs, or tremors, were found in a majority (25 out of 33). Impaired balance and coordination were common; almost half had difficulties with bathing or dressing. Educational support was commonly provided.

Neurologic abnormality is more common in patients with apoenzyme defects than those with defects in cobalamin synthesis [30–32]; however, abnormalities in central nervous system (CNS) function may be seen in any patient with methylmalonic acidemia (Figures 3.7, 3.8, and 3.9). Dystonia and weakness profound enough to lead to a wheelchair-bound state has been observed in methylmalonic acidemia [33]. This has been associated with neuroradiologic evidence of abnormality in the basal ganglia, which has frequently been encountered in disorders of propionate metabolism [34–36]. In patients imaged by computed tomography (CT) or MRI, specific lesions are regularly seen in the basal ganglia (Figures 3.8 and 3.9), even in patients with no relevant clinical findings. Lesions in the globus pallidus are regularly seen in mut^0 and mut^- patients, but they are also seen in cobalamin-responsive patients [34–40]. At the extreme, a syndrome of metabolic stroke has been reported with what looks like infarction of the basal ganglia, especially the globus pallidus, and

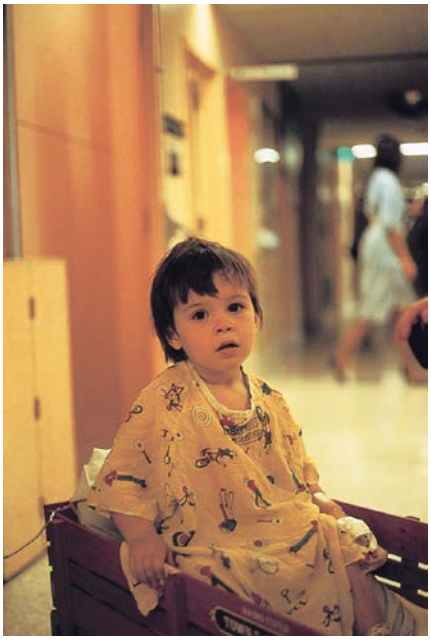

Figure 3.7 TJ: A boy with B_{12}-responsive methylmalonic acidemia of the Cbl A type. He only had the initial severe acidotic episode, but his behavior was sufficiently unusual that he had been characterized as autistic.

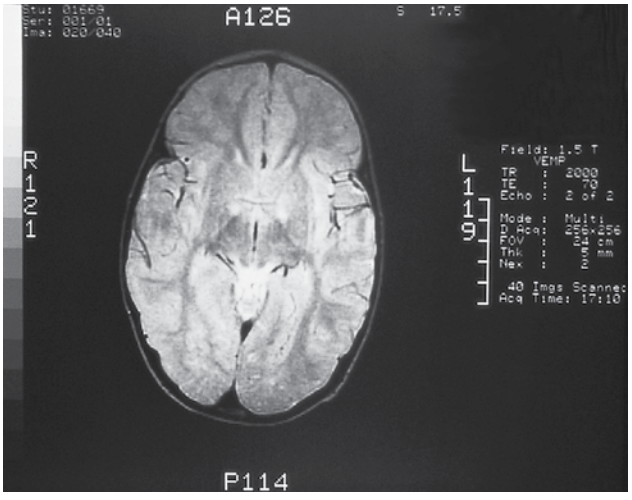

Figure 3.8 MRI of the brain of TJ revealed increased intensity of T_2 signal in the basal ganglia.

acute dystonia [33, 35, 41]. Diffusion-weighted imaging (DW-MRI) has been reported [42] to aid in the detection of acute basal ganglia infarction and the distinction of acute from chronic changes. The authors emphasized that basal ganglia stroke can occur even after many years without acute metabolic imbalance. Decreased white matter attenuation on CT and high T_2 signal on MRI may be seen early [32, 39, 43]. This may progress to cerebral atrophy and quadriparesis [33]. Harting and colleagues [44] have called attention to a spectrum of changes in the MRI of patients with methylmalonic, in addition to the characteristic lesions of the basal ganglia (pallidum). Their patients had

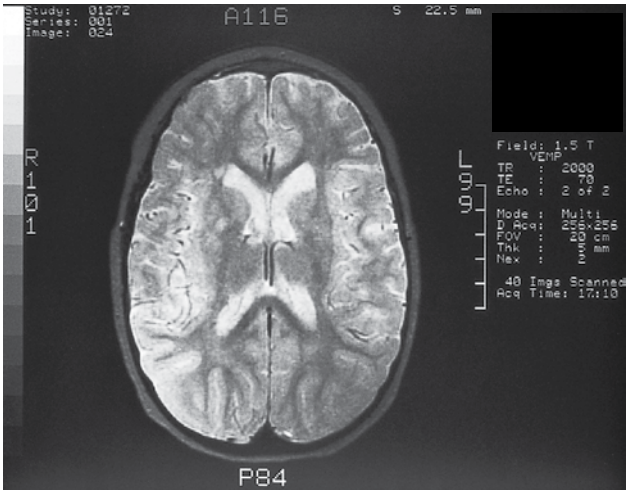

Figure 3.9 MRI of CH, a patient with methylmalonyl CoA mutase deficiency. There was a diffuse pattern of abnormal signal intensity in the cerebral hemispheres and focal areas of abnormal signal in the basal ganglia.

delayed maturation of the brain, immature gyral patterns, incomplete operculation, and white matter disorder. Changes were found in the brain stem and cerebellum in all of their children, as well as T_2 hyperintensity and volume loss. Some patients have had convulsions, and abnormalities of the electroencephalogram (EEG) are more common [21, 33]. In one patient, who died at 6 days with hyperammonemic coma and ketoacidosis, there was a burst suppression pattern [45]. Neuropathology in this patient revealed diffuse gliosis in the white matter, Alzheimer type II cells, and cerebellar hemorrhage.

In some patients, abnormal neurologic signs increased with age [32, 33]. Among patients surviving longer, late effects, including CNS abnormalities, are becoming apparent [32]. We have reported a mut^0 young adult who developed weakness in her teens and became wheelchair-bound. She had acute involuntary spasms of the legs and more general spasms resembling myochymia. Cognitive function was not impaired. Among late effects, blindness developed in a 21-year-old patient two months before he died; he had been in a wheelchair but rode horses and could sail a yacht and drive farm equipment before developing optic atrophy [46]. Optic atrophy is becoming increasingly recognized as a late effect of this disease [47].

Some patients have hepatomegaly. Liver function tests are normal. Renal functional impairment has been reported [1] and we have observed chronic renal tubular acidosis [48]. Hyperuricemia is usually present, a consequence of competition for its renal tubular excretion. Urate nephropathy and renal failure have been reported [49]. Tubulointerstitial nephritis has been reported in four biopsied patients of 15 reported with renal disease [50]. End-stage renal disease requiring dialysis and/or transplantation has been observed, as another late complication [35, 51, 52].

Pancreatitis has been reported in a variety of organic acidemias [53]. Among the patients, methylmalonic

acidemia was particularly prominent. Five of nine patients had methylmalonic acidemia; of these, two died. One of our adult patients with mut^0 disease died of acute hemorrhagic pancreatitis.

Transient thrombocytopenia has been observed in infancy. Neutropenia is a regular occurrence except in the case of successful treatment and reduction in the accumulation of MMA in body fluids. Anemia may occur, especially in the first month of life. Recurrent infections are common.

Chronic moniliasis is highly relevant to metabolic control. High levels of methylmalonate and other intermediates that accumulate when patients are out of control inhibit the maturation of hematopoietic cells and also of T cells, so the T-cell number is low. The response of T cells to Candida is also specifically altered when levels are high [26]. When metabolite levels are lowered by treatment, skin lesions disappear and T-cell responsiveness to Candida returns. Osteoporosis has been found regularly and we have observed femoral and tibia fractures.

Patients with B_{12} responsiveness, in both the Cbl A and Cbl B complementation groups not only had milder disease, they presented later than those with mut^0 or mut^- disease [30]. Some 80 percent of mut^0 patients presented within the first week of life; 42 and 33 percent, respectively, of Cbl A and Cbl B patients presented this early. The mut^0 patients were predominantly dead or severely impaired at follow up. Most died within two months of diagnosis. Most of the Cbl A and Cbl B patients were alive at follow up.

At least four successful pregnancies have been reported in women with methylmalonic acidemia [54, 55] despite evidence of renal impairment. One was mut^- and one B_{12}-responsive. As predicted, levels of MMA decreased dramatically as the fetus grew [55]. These experiences documented that MMA is not teratogenic.

Some patients with MMA have been clinically normal. Presumably, these individuals are mut^- variants with a considerable level of activity *in vivo*. So-called "benign methylmalonic acidemia" has been reported in at least nine clinically normal individuals [56, 57], eight of them identified through routine neonatal screening [56]. Some of these patients may excrete quite large amounts of MMA. In the Quebec program of screening neonatal urine for MMA [57], a follow-up study of 122 individuals with MMA excretion over 1400 mmol/mol creatinine indicated that MMA excretion had resolved by one year in 65 and in ten more over 15 months to seven years; so, a majority were transient. Of the rest, 13 were symptomatic and 22 asymptomatic. MMA levels in blood and urine were appreciably higher in the symptomatic patients. Careful study of the asymptomatic patients revealed one to be mut^- and the rest undiagnosed. All of the asymptomatic patients were found to be clinically and cognitively normal at follow up. Programs of neonatal screening are finding patients with MMA in appreciably greater numbers than were evident from experience with illness presentations. In the California pilot study of screening by tandem mass spectrometry (MS/MS), a newborn population screening of 309,074 yielded eight methylmalonic acidemia patients. This prevalence rate of 1 in 3800 represented the third most common disorder detected and the only prevalent organic acidemia. Some of these patients might represent those who might have died undiagnosed, but some of them are likely to represent more benign disease. Clues from the Quebec study [57] indicated that the benign patients are likely to have no urinary metabolites of propionate, such as hydroxypropionate or methylcitrate, and many excrete malonic acid in amounts of 60–227 mmol/mol creatinine.

In addition to the differential diagnosis of methylmalonic acidemia shown in Table 3.1, there are a number of patients of variable, atypical phenotype in whom the molecular nature of the disease has not been defined [58, 59]. Most have had appreciably lower levels of MMA than in a classic patient and activities of the mutase enzyme are normal. Treatment with B_{12} had no effect, and protein restriction may not decrease the excretion of MMA. Some, but not all, have also excreted malonic acid. These patients have not had a crisis of ketoacidotic metabolic imbalance. They have usually been investigated because of failure to thrive or developmental delay. Some have had athetoid movements, myopathy, and ophthalmoplegia or pyramidal tract signs [58]. Two siblings developed renal tubular acidosis with hypercalciuria, one of whom developed nephrocalcinosis [59].

GENETICS AND PATHOGENESIS

Each of the forms of methylmalonic acidemia is determined by a rare autosomal recessive gene. Complementation studies [60, 61] have indicated that there are at least four distinct forms of methylmalonic acidemia. Furthermore, the mut apoenzyme defect group is heterogeneous. Mut^0 patients have no activity of the enzyme, while mut^- patients have a spectrum of residual activity. Heterozygote detection by enzyme analysis may be unreliable.

Prenatal detection of methylmalonic acidemia has been accomplished by assay of the activity of methylmalonyl CoA mutase in cultured amniotic cells [62]. The diagnosis has also been made chemically by assay of the maternal urine for MMA [63], but this may not be reliable until quite late in pregnancy. Rapid, efficient chemical diagnosis can be made by direct analysis of the amniotic fluid for methylcitric acid or MMA using stable isotope dilution methodology and gas chromatography-mass spectrometry (GCMS) [63–66]. Fully-automated high throughput assay has been reported [67]. In a family in which the mutation is known, its determination can be used for prenatal diagnosis, and for heterozygote detection. An infant with defective adenosylcobalamin synthesis was diagnosed prenatally and effectively treated with cobalamin prenatally [68].

The diagnosis of methylmalonic acidemia is most readily made by assay of the urine for MMA (Figures 3.10 and 3.11). Screening tests are now seldom used, and the

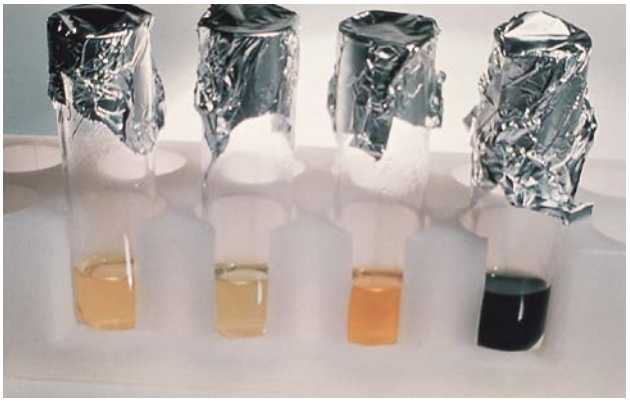

Figure 3.10 Colorimetric test of urine for MMA. The dark green color which develops in the presence of p-nitroaniline has been used for screening.

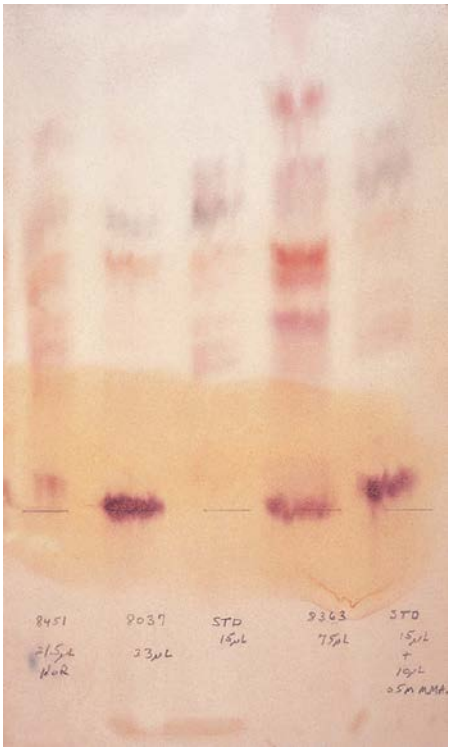

Figure 3.11 High-voltage electrophoresis of the urine. The amino acids have been separated and stained with ninhydrin and then the paper was overstained with Fast Blue B giving a purple band at the origin in the presence of MMA. Numbers 8037 and 8363 were patients with mut^0 methylmalonic acidemia.

diagnosis is usually made by organic acid analysis of the urine with GCMS. The amounts of MMA excreted are enormous. Excretion of a gram a day by a tiny infant is not unusual. Normal individuals excrete less than 5 mg per 24 hours, amounts that are undetectable in the usual assays. A comparison of the amounts of methylmalonate excreted in patients with various forms of methylmalonic aciduria is shown in Table 3.2.

Table 3.2 Excretion of MMA

Clinical status	Amount excreted (mmol/mol creatinine)
Normal	0–2
Mut^0; presentation	3000–13000
Mut^0; steady-state	200–2000
B_{12}-responsive; presentation	2000
B_{12}-responsive; steady-state	90–300
B_{12}-deficient infant	4500–5700
Transcobalamin II deficiency	600
Cobalamin C, D	270
Atypical-normal mutase	200
Succinyl CoA ligase	80–120
Methylmalonyl CoA epimerase deficiency	50–300

MMA, undetectable in the plasma of normal individuals, is present in patients in concentrations of 200–2500 μmol/L. The concentrations of MMA in the cerebrospinal fluid (CSF) may equal that of the plasma. In patients with cobalamin deficiency, concentrations in CSF tend to be much higher than in plasma [69]. Propionic acid also accumulates in the plasma of patients with methylmalonic acidemia [70], and 3-hydroxypropionate [71] and methylcitrate [72] are found in the urine. The administration of isoleucine, threonine, valine, or methionine results in the formation of MMA [73].

The diagnosis of methylmalonic acidemia is increasingly made by MS/MS, not only in programs of newborn screening, but by quantitative analysis of acylcarnitine profiles of plasma (Chapter 1). Answers are more rapidly available in an emergency situation than by GCMS of urinary methylmalonate. The quantification of urinary methylmalonate remains the best approach to monitoring the effectiveness of therapy. Electrospray MS/MS of urine in positive and negative modes has been reported as a rapid approach to diagnosis of a variety of inborn errors of metabolism [73]. Separation of succinic and MMAs was not achieved, but the disparity in amounts excreted in illness indicated utility in the diagnosis of methylmalonic acidemia. A liquid chromatography (LC)-MS/MS method with a deuterated internal standard has been reported [74] that is rapid and accurate and correlated well with GCMS in the analysis of the same samples; it can be used for plasma or urine. Methodology for the analysis of methylmalonic acidemia is of broad applicability in addition to the diagnosis and management of inborn errors of metabolism, because elevated plasma concentration of MMA that responds to B_{12} is the best indicator of tissue deficiency of cobalamin [75].

All patients with methylmalonic acidemia have defective activity of methylmalonyl CoA mutase (Figure 3.1), the enzyme that catalyzes the conversion of methylmalonyl

CoA to succinyl CoA (see Figure 2.1). This enzyme lies on the direct degradative pathway for isoleucine, valine, threonine, and methionine. All of these amino acids have been shown to be major sources of methylmalonate in patients. On the other hand, lipids, although metabolizable via this pathway, do not contribute in measurable fashion to urinary MMA [76].

Apoenzyme defect was first demonstrated in the liver of four patients who died, by measuring the conversion of ^{3}H-methylmalonylCoA to ^{3}H-succinylCoA [14]. In the mut^{0} group, mutase activity in cultured fibroblasts or tissues is undetectable even in the presence of adenosylcobalamin [77–79]. In the mut^{-} group, some residual activity is present. Heterogeneity has been demonstrated in the mut^{0} group because some patients are cross-reactive material (CRM)-negative, and some have reduced amounts of CRM [80]. Some patients in the mut^{-} group may have much later and much milder clinical presentations. Enzyme activity of 2–75 percent of control was associated with CRM of 20–100 percent of control [80]. In studies of labeled enzyme synthesis, some mut^{0} patients made unstable enzyme, which disappeared, while most made no detectable enzyme [81]. All mut^{-} patients made detectable newly synthesized enzyme.

The study of fibroblasts of patients with B_{12}-sensitive methylmalonic aciduria [82] clarified the nature of these disorders. The content of adenosylcobalamin is reduced, and the cells cannot convert ^{57}Co-hydroxycobalamin to ^{57}Co-adenosylcobalamin [83].

A simplified test for the overall enzymatic block at the mutase step is to test the conversion by cultured fibroblasts of ^{14}C-propionate to $^{14}CO_2$ [84]. Assessment of ^{14}C-MMA oxidation permits distinction of MMA from propionic acidemia. The extrapolation of this assay to the incorporation of ^{14}C-propionate into acid precipitable material has simplified the procedure [85]. This has been employed in studies of complementation among the inherited methylmalonic acidemias. Patients responsive to B_{12} were promptly subfractioned into two complementation groups, designated Cbl A and Cbl B [60, 61, 86, 87]. The Cbl A variants synthesize adenosylcobalamin normally from ^{57}Co-hydroxycobalamin and adenosine triphosphate (ATP) under reducing conditions [83]. In the Cbl B variants, adenosylcobalamin synthesis is defective under these conditions, and the defect has been shown to be in the adenosyltransferase [88, 89]. Patients with defects in cobalamin synthesis generally present later than those with apoenzyme defects, and most survive the illness once diagnosed. Among the Cbl A patients, a clinical response to B_{12} is regularly seen, while in Cbl B patients only half respond to B_{12} with a decrease in the amounts of MMA in body fluids, suggesting that there is a complete block in the unresponsive patients.

The cDNA for methylmalonyl CoA mutase was originally obtained from human cDNA hepatic libraries; it was used as a clone to localize the gene to human chromosome 6q12–21.2 [15, 16]. A highly informative restriction fragment length polymorphism (RFLP) at this locus, a *Hin*dIII polymorphism, has been used for heterozygote detection, prenatal diagnosis, and linkage analysis. By now, approximately 200 mutations have been identified in the *MUT* gene [17–19, 90, 91].

Four mutations in mut^{-} cells, all of which exhibited interallelic complementation, clustered near the carboxyl terminus of the protein. These missense mutations (R664W, G648D, G630E, and G626C) (respectively, arginine to tryptophan, and glycine to aspartic acid, glutamic acid, and cysteine) were close to another mutation (G717V) (glycine to valine) in the region that appears likely to be the cobalamin-binding domain. The enzyme in these cells could be stimulated *in vitro* by very high concentrations of hydroxycobalamin. R694W was also found in the mut^{-} phenotype [18]. The enzyme specified by G717V mutation was shown to have a very high Km for adenosylcobalamin. The enzyme bearing the G648D mutation also had a high Km. It is of interest that six of seven mutations described in this area involved substitution for a glycine residue, suggesting altered secondary or tertiary structure [92]. Among mut^{0} patients, mutations near the amino terminal of the protein eliminated enzyme activity entirely [93, 94]. G717V has been observed to be common in black Americans [92, 93]. Among Japanese, E117X was found in every patient reported [95]. At least one mutation has been reported, an N terminal deletion which interfered with processing of the enzyme, such that it was not taken up by the mitochondria [96]. Gene transfer has been employed [95] as a substitute for complementation in the distinction of mut from Cbl phenotypes. In 25 predominantly Spanish patients, frame-shift mutations were prevalent [97]. Transfer of a normal mutase cDNA clone corrected activity as measured by ^{14}C-propionate assay. Transfer into Cbl fibroblasts had no effect on activity. In European patients with the Cbl A disease and a severe clinical phenotype, R145X was found in 43 percent of mutant alleles [98]. In two patients with the Cbl B disease, I96T and G97fs mutations were found [97]. A homozygous nonsense mutation R47X was found in a patient with mild methylmalonic aciduria in the epimerase gene (*MCEE*) [8]. In one group [97] of Cbl A patients, all mutations led to truncated proteins [97].

It may be relevant to pathogenesis that mitochondrial dysfunction has been observed in mut methylmalonic acidemia [99]. In Mut knockout mice, mega mitochondria were found in hepatocytes early in life, and there was respiratory chain dysfunction with diminished cytochrome oxidase activity and low intracellular glutathione. Similar findings were observed in the liver of a patient who had undergone liver transplantation. The mice also developed tubulointerstitial renal disease. A patient with Cbl A disease responsive to B_{12}, developed multiorgan failure and died; multiple defects in OXPHOS were found in the liver [100]. The terminal episode was ushered in by the sudden onset of optic atrophy.

TREATMENT

Patients with methylmalonic acidemia should first be tested for responsiveness to B_{12}. This is important for a majority of those responding have survived, while the majority of the unresponsive, who were detected as a result of ketoacidotic illness, have not survived [30] or have survived with major neurologic disability.

In patients with B_{12}-responsive methylmalonic acidemia, excretion of MMA in the urine is significantly decreased by the administration of pharmacologic doses of cyanocobalamin [3, 101]. We have employed the method of admission to the general clinical research center (GCRC) and measurement of total MMA excretion for five days, the first two control days and the next three reflecting daily injection of 1 mg of hydroxycobalamin or cyanocobalamin. This method has elucidated the true status in patients in whom the results of casual specimens under varying conditions in the clinic, or in acute admission to hospital, are confusing. A similar protocol has recently been published [17]. A decrease of mean urine or plasma MMA concentrations of 50 percent was deemed responsiveness.

The correlation of B_{12} responsiveness with prognosis is clear [30]; all but four of 25 children who responded to B_{12} were alive, while 11 of 20 who did not respond to B_{12} died. The first B_{12}-responsive patient was well at nine years when reported [102], and was 14 at the most recently reported follow up.

Those who respond are treated with B_{12} in doses sufficient to keep concentrations of MMA minimal. B_{12}-responsive patients may do very well with modest protein restriction, growing and developing normally over the long term and tolerating childhood illnesses [103]. Most Cbl A patients can be expected to respond clinically to B_{12}, while about half of the Cbl B patients respond [30]; mut^0 and most mut^- patients have not responded despite *in vitro* evidence of responsiveness. Nevertheless, some mut^- patients will respond to B_{12}; so they all should be tested [104].

Patients who do not respond to B_{12} are treated with a diet designed to keep the precursors of MMA at a manageable level [23, 104, 105]. This is complicated because isoleucine, threonine, methionine, and valine are all essential for normal growth and development. Therefore, optimal therapy consists of a diet containing the minimal requirements of these amino acids for optimal growth and no more. The rest of the calories can be made up of a diet containing fat and carbohydrate, with or without other amino acids. The amount of protein necessary to accomplish this must be individualized. Under conditions of limited intake of protein, caloric intake must be generous. We have found that alanine supplementation is useful in this disorder and may replace a mixture of amino acids [106]. The management of such a patient is not easy. It requires enormous commitment on the part of parents, physicians, and nutritionists. Furthermore, treatment must be monitored by periodic quantitative assay concentrations of MMA to ensure optimal control, and of plasma amino acids to ensure the avoidance of protein malnutrition. Nevertheless, it may be successful. The reward in normal development may be high. In 25 years of experience with 66 patients with methylmalonic acidemia, Ney and colleagues [105, 107] pointed out that 29 of 50 B_{12}-unresponsive patients died, most of them prior to 1985, and only three after 1985. Of 21 living patients, most were judged to have had good or very good results. Treatment after 1985, reflected very rigid restriction of protein [104, 107], the addition of carnitine and the use of metronidazole [108, 110]. Not only was survival improved, but the number and severity of metabolic decompensations were decreased [107].

Propionic acid is synthesized by intestinal bacteria, and this may be an important source of propionate and methylmalonate in these patients [110]. Treatment with neomycin or metronidazole may reduce levels of propionic and MMAs in body fluids [108–110]. Doses of metronidazole have ranged from 10 to 20 mg/kg per day and have been divided into three doses. Neomycin has been used in a dose of 50 mg/kg. Other antibiotics, such as bacitracin, paromycin, clindamycin, or vancomycin, may be useful in acute situations. Lincomycin was not effective [110]. In our experience, intermittent antibacterial therapy has been useful, suggesting that clonal populations of propionate-forming bacteria may be intermittently present in some patients. An effect of antibiotic treatment on metabolite accumulation may be especially useful during a crisis of metabolic decompensation. A sudden increase in MMA excretion unaccompanied by dietary change or stimulus for catabolism may suggest a bacterial source and an argument for neomycin or metronidazole.

An increase in the excretion of metabolites of propionate during fasting, suggests the mobilization of odd-chain fatty acids from lipid stores [111]. The therapeutic implication is the avoidance of fasting and the use of intravenous calories when the oral route is not available.

In the management of the acute ketoacidotic crisis, a program of aggressive fluid and electrolyte therapy is essential as set out for propionic acidemia (Chapter 2). In addition, in methylmalonic acidemia, advantage can be taken of the very effective excretion of methylmalonate by the kidney [109], which is much more efficient than peritoneal dialysis, by aggressive intravenous hydration (150–200 mL/kg of water containing 10 percent glucose and initially isotonic $NaHCO_3$ until the acidosis is corrected). Anabolism may be promoted by the use of insulin and glucose, or the acute use of growth hormone. Vomiting may be relieved with ondansetron (0.15 mg/kg over 15 minutes, intravenously, up to three times a day).

Carnitine has been a useful adjunct to chronic maintenance therapy, removing propionyl groups as carnitine ester [113, 114] and diminishing the propensity of these patients to abnormal ketogenesis [114]. We have found parenteral carnitine in doses of 300 mg/kg very useful in the acute crisis.

Human growth hormone may be useful in adjunctive therapy in this and other organic acidemias [115]. Promotion

of anabolism may diminish the propensity for catabolism, and thus the acute catabolic response to infection, stress, or protein intake. In our hands, protein requirements have increased without increase in metabolite excretion. Growth has been rewarding, as well as increase in lean body mass and decrease in adipose tissue.

The fact that so many patients with mut^0 disease die in infancy and that survivors have so often had severely impaired mental development [30, 31] has led to consideration of transplantation of liver [32, 116, 117]. Our experience [32] has indicated that liver transplantation does not halt or reverse relentless progression to renal failure. Most such patients will have had evidence of renal impairment at the time liver transplantation is considered. It makes sense to treat such a patient with combined transplantation of liver and kidney. That decision is not so clear in the case of an infant.

Our experience with liver transplantation also indicates that the procedure does not stop the progression of late onset neurologic disease [32], although it completely does away with recurrent attacks of ketoacidotic metabolic imbalance. High concentration of MMA in the CSF does not decrease with liver transplantation (Nyhan, unpublished data) [117]. It is of interest that high CSF concentrations of MMA have also been observed in patients with cobalamin deficiency [118]. Also, transplantation of the liver did not prevent the occurrence of infarction of the basal ganglia during an episode of pneumonia unassociated with metabolic imbalance [119]. Neurologic deterioration as manifested by a cerebellar stroke was also observed in a 5-year-old boy who had a combined liver kidney transplantation [120]. Combined transplantation of the liver and kidney appears to be a better approach [120, 121]. Tacrolimus toxicity has been pointed out to be a confounding feature in the interpretation of neurologic complications, but this is treated by reducing dosage. Metabolic decompensation does not generally occur following liver transplantation.

The treatment of hyperammonemia in the acute crisis of infantile methylmalonic acidemia has been expanded by the use of intravenous carbamylglutamate in this disease as well as in propionic acidemia (Chapter 2) [122, 123, 124]. The adult dose was 150 mg/kg, followed by 100 mg/kg daily. Carbamylglutamate has also been used to treat hyperammonemia in N-acetylglutamate synthetase deficiency.

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4

Cobalamin C, D, F, G diseases; methylmalonic aciduria and variable homocystinuria

MAJOR PHENOTYPIC EXPRESSION

Megaloblastic anemia; failure to thrive; developmental delay; excretion of homocystine and methylmalonic acid; defective activities of methylmalonyl CoA mutase and/or methionine synthase; and mutations in the transcription coregulator *HCFC1*.

INTRODUCTION

Patients with methylmalonic aciduria and homocystinuria have defective metabolism of cobalamin to both cofactors, methylcobalamin and deoxyadenoxylcobalamin [1–4]. Accordingly, the activities of methionine synthase and methylmalonyl CoA mutase are defective (see Figure 4.1). Patients with impaired synthesis of methylcobalamin and deoxyadenosylcobalamin fall into two distinct complementation groups designated Cbl C and Cbl D. Another group of patients designated Cbl F have defective transport of free cobalamin out of lysosomes. In Cbl G disease, defective activity of methionine synthase results from mutations in the methionine synthase gene [5]. The differential diagnosis of methylmalonic acidemia and homocystinuria is given in Table 4.1.

The Cbl C disease is the most common, and its clinical picture is heterogeneous, but a majority of patients have early onset disorder with major dysfunction and a median age at death of two months [3, 6].

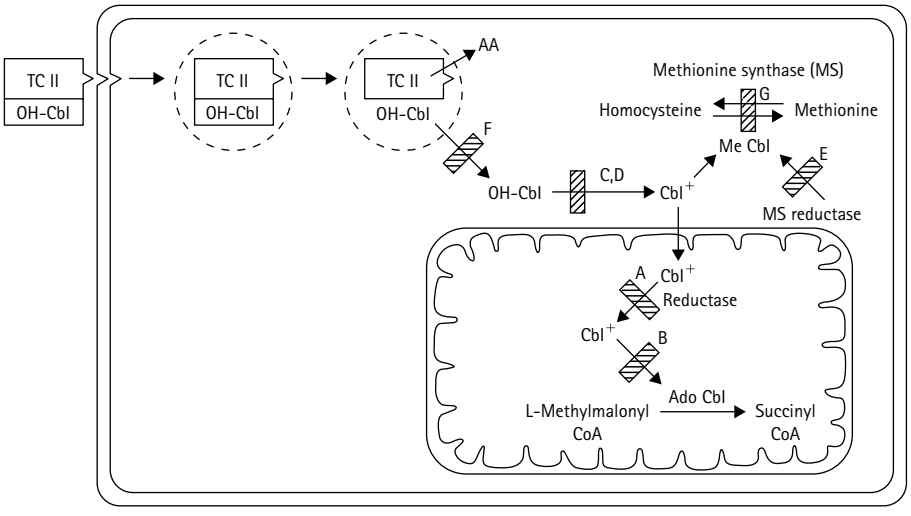

Figure 4.1 Cobalamin transport and metabolism sites of the defects in complementation groups A to G.

Table 4.1 Differential diagnosis: methylmalonic acidemia and homocystinuria

Disorder	Methylmalonic aciduria	Homocystinuria	Methionine increase	Serum B_{12} low
Cobalamin (Cbl) C	+	+	0	0
Cobalamin (Cbl) D	+	+	0	0
MMA mutase apoenzyme (Mut^0, Mut^-)	+	0	0	0
Cystathionine synthase	0	+	+	0
Methylenetetrahydrofolate reductase	0	+	0(↓)	0
Cobalamin (Cbl) E, G	0	+	0	0
Cobalamin (Cbl) F	+	+	0	0
Cobalamin X	+	+	0	0
B_{12} deficiency	+	+	0	+
Gastrointestinal surgery	+	±	0	+
Autoimmune-multiple endocrine deficiency, antibody to parietal cells	+	±	0	+
TC II deficiency	+	±	0	+
Cobalamin-enterocyte malabsorption-Immerslund-Grassbeck	+	±	0	+

The disease gene (*MMACHC*) has been cloned [7] and over 148 mutations have been identified [3, 6–8]. The most common mutation c.271dupA accounted for over 50 percent of southern European and French-Canadian patients [6, 9]. This and two other mutations, c.394C>T and c.331C>T accounted for most of the populations studied; indicating that rapid search for these three mutations in infants, detected by newborn screening, could yield presymptomatic diagnosis and the more rapid introduction of treatment.

Patients with the Cbl D defect may have isolated deficiency of either methylcobalamin synthesis (variant 1), or adenosylcobalamin synthesis (variant 2) [10, 11], as well as the combined synthesis originally described. The gene was localized to chromosome 2q23.2, and a candidate gene named *MMADHC* was identified [12]. The predicted MMADHC protein had sequence homology with a bacterial ATP-binding cassette transporter. It is now clear that these three different types of Cbl D mutations are dependent on where in the gene the N-terminal occurs [13]. Null mutations for the N-terminal to methionine 116 cause isolated methylmalonic acidemia (Cbl D-MMA), and deficiency of AdoCbl, null mutations across the C-terminus (p.Y140–R250) cause combined methylmalonic aciduria and homocystinuria (Cbl D MMA/HC) due to deficiency of AdoCbl and MeCbl; and missense mutations in a conserved C-terminal region (pD246–L259) cause isolated homocystinuria (Cbl D –HC).

A clinical picture like that of severe Cbl C disease has recently [10] been found to result from mutations in a global transcription coregulator. The gene *HCFC1* is located on the X chromosome. Five distinct mutations were found, the most common c.344C>T in 9 patients; c.218C>T in 3, and c.343G>A and c.202C>G in one patient each.

CLINICAL ABNORMALITIES

The clinical manifestations of Cbl C disease often begin with megaloblastic anemia and failure to thrive (Figure 4.2) [1, 2]. Death may occur within the first six months of life [1, 3], and there may be overwhelming illness starting in the first days of life. Some patients have seizures; some have microcephaly. Lethargy and/or irritability are prominent. Patients may be difficult to feed. Patients with onset later than the early months of life have had predominantly neurologic presentations. Anorexia, irritability, or fatigue may be seen, as well as myelopathy or dementia. Hematologic examination is like that of pernicious anemia

Figure 4.2 JA: A four-year-old boy with Cbl C disease. He looked quite good. His magnetic resonance images are shown in Figures 4.3 and 4.4. (Images were kindly provided by Dr GM Enns of Stanford University).

with hypersegmented polymorphonuclear leukocytes, and sometimes thrombocytopenia, as well as the megaloblastic anemia. One patient [14] had severely impaired mental development and megaloblastic anemia; he died at seven years of age. As the numbers of patients recognized with Cbl C disease has increased, clinical heterogeneity has become apparent [4]. Retinal degeneration has been reported [15], as well as pigmentary retinopathy, which may aid in the clinical diagnosis. Another infant presented at eight months of age with hypotonia, failure to thrive, and macrocytic anemia [16]. He did not appear to see or hear, and visual and auditory evoked potentials were abnormal. Infants with neonatal onset who survive the initial episode may have metabolic decompensation during intercurrent illness, as in other organic acidemias. Early onset patients accounted for 88 per cent of patients [3]. Plasma total homocysteine was higher and methionine lower in this group of patients [3].

A 30-year-old patient was reported [17] who presented first at 12 years of age with fatigue, ataxia, and mild incontinence, indicating involvement of the spinal cord. Seven years later, she developed peripheral nerve disease. A relapsing-remitting course suggested multiple sclerosis. At 24 years, she had deep vein thrombosis and lost the ability to walk. Six months later, she required intensive care. A 34-year-old sister with the same metabolic defect was well. Neither had hematologic abnormalities. Another patient with late onset Cbl C deficiency presented with psychosis, anorexia, weight loss, seizures and ataxia [18]. Manifestations resolved with treatment with hydroxocobalamin and folate. Other late onset patients have had characteristic psychiatric/behavior problems and myelopathy [3]. Late onset patients accounted for 2 per cent of the total [3]. A small group of neonates with methylmalonic acidemia and homocystinuria of the Cbl C group have presented with microangiopathy, anemia, and a hemolytic-uremic syndrome. All have died early in life [19].

Cutaneous manifestations consistent with a diagnosis of acrodermatitis enteropathica were reported in two infants with Cbl C disease [20]. Lesions were erythematous, superficially erosive, desquamative, and hyperkeratotic. There was associated cheilosis and perioral erosions. Lesions of this type have been attributed to nutritional deficiency in many inborn errors under treatment, but these patients presented with skin lesions at 9 days and 19 days, before nutritionally restrictive therapy had begun. On the other hand, both had very low levels of methionine in plasma: 10 μmol/L and 1 μmol/L; so, this could still represent deficiency of an essential amino acid.

Among those with Cbl C disease surviving early infancy, neurologic manifestations have been prominent. Impaired mental development has been the rule [4]. Microcephaly, nystagmus, visual impairment, and retinopathy have been prominent features. Progressive neurodegenerative disease was reported [21], despite early treatment with hydroxocobalamin and improvement in the concentrations of metabolites. The patient had presented at 9 days of life and was treated with hydroxocobalamin within the next 2 weeks. She developed choreoathetosis and brisk reflexes at 13 months and seizures at 15 months. Acute stroke with coma has also been observed in this disease [22], although not with the frequency seen in cystathionine synthase deficiency.

In a series of 41 Portuguese and Italian patients [6], 36 patients had early onset disease presenting in the first year of life, and five had later onset disease. Prenatal onset abnormalities, such as microcephaly and intrauterine growth impairment, were seen in a number of patients. All of the early onset patients had failure to thrive, feeding difficulties, and episodes of acidosis, thrombocytopenia, leukopenia, or anemia. Three patients in this series had a hemolytic-anemic syndrome. With time, nearly all had cognitive/developmental delay. Late onset more attenuated patients had mild to moderate cognitive impairment, seizures, and corticospinal tract signs. Some late onset patients have presented with neuropsychiatric symptoms. Association with vasculopathy and mitochondrial electron transport chain dysfunction has also been observed. Late onset patients in general have had better survival, responded to treatment, and had fewer neurologic sequelae [23] than early onset patients. Two siblings had late onset hemolytic-uremic thrombotic microangiopathy [24]. They were effectively treated with hydroxycobalamin.

Neuroimaging [3, 24, 25] revealed evidence on magnetic resonance imaging (MRI) of diffuse edema and dysmyelination of white matter at presentation, and volume loss of white matter with time, and communicating hydrocephalus (Figures 4.3 and 4.4). Normal findings

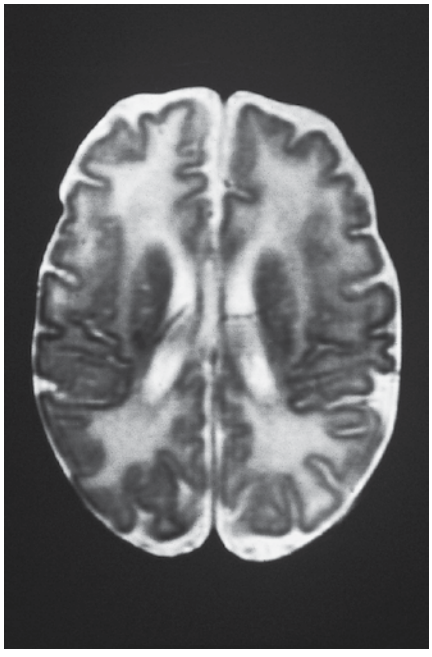

Figure 4.3 MRI of the head of a 5-week-old infant with Cbl C disease. Abnormal signal was consistent with white matter disease. (Images were kindly supplied by Dr GM Enns of Stanford University.)

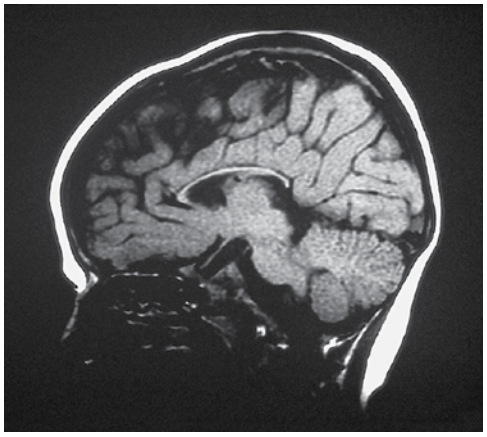

Figure 4.4 MRI of the brain of the same patient at 23 months. By this time, there was extensive paucity of myelination and diffuse atrophy. The corpus callosum was very thin. Neurologic progression occurred despite therapy with hydroxocobalamin. (Images were kindly provided by Dr GM Enns of Stanford University.)

were observed in 19 per cent of patients. Hydrocephalus occurred only in the infantile onset patients [3]. Electroencephalogram (EEG) may show epileptiform abnormalities [25]. Evoked responses display increased latency and prolonged conduction [17, 23, 25]. In the patient with the neurodegenerative picture, there was prominent involvement of the globus pallidus on MRI and clear evidence of progression. Neurologic progression and cerebral atrophy were observed despite therapy with hydroxocobalamin. The neonatal MRI was normal; that of one year revealed white matter loss, ventricular enlargement, and normal basal ganglia. At 15 months, both globi were hyperintense. Lesions in the basal ganglia have been observed in methylmalonic acidemia of the mut^0 and mut^- types in Cbl A and Cbl B disease, and in propionic acidemia. We have also observed similar involvement of the globi pallidi in transcobalamin II deficiency. Multiple small infarcts of the basal ganglia were found in the basal ganglia of a patient with Cbl C disease who died at 22 months [18].

Only two patients have been reported with Cbl D disease; they were brothers, neither of whom was anemic [2]. The older one had impaired mental development and psychosis, and he had abnormalities of cerebellar and spinal cord function, including ataxia. His two-year-old affected brother appeared well at report at two years of age. Thromboembolic complications may be observed, as in cystathionine synthase deficiency (Chapter 18).

Five patients have been reported with Cbl F disease [26, 27]. Deep tendon reflexes were accentuated and there was an intention tremor. The first two presented within the first 2 weeks of life with stomatitis, failure to thrive, and hypotonia [26]. Seizures [26, 28] were observed, as was developmental delay. There were no hematologic abnormalities in the first patient, but macrocytosis, hypersegmented polymorphonuclear leukocytes, and even pancytopenia have been observed. One infant died suddenly despite a good biochemical response to cobalamin.

Patients with Cbl G deficiency, like those with Cbl C, have megaloblastic anemia and neurologic abnormalities with undetectable activity of methionine synthase. Patients were admitted to hospital within the first year of life with anemia and very high levels of homocysteine, while those of methionine were low, and B_{12} levels were normal.

The clinical picture of the newly discovered Cbl X disease [13] resembles that of a severe Cbl C disease. In three patients, manifestations such as microcephaly had a prenatal onset. In the others, manifestations began at four months or less. Infantile spasms and hypsarrhythmia were seen in three patients. All the rest had seizures. Severe delay or absence of development was the rule. One had a gyral cortical malformation, undescended testis and megacolon. One had hypospadias.

The excretion of methylmalonic acid in the urine was statistically significantly higher in patients with Cbl X ($p<0.01$) than with Cbl C [13]. Plasma concentrations of homocysteine were not statistically different, but the range, both higher and lower, was greater for the homocysteine in plasma; normal levels were recorded in four. The most commonly defective abnormality of intercellular cobalamin metabolism is Cbl C [29]. Elevated levels of methylmalonic acid and homocysteine and decreased production of methionine are the biochemical hallmarks of the disease. Macular and retinal degeneration are unique features of Cbl C disease [30]. Deterioration of visual activity was documented [31]. Maculopathy, retinopathy, nystagmus, strabismus and optic atrophy were commonly encountered. Among the clinical manifestations, an atypical hemolytic uremic syndrome is uncommon [32]. It occurs generally within the first 30 days of life. It has also been reported [33] in a 20-year old man with malignant hypertension and renal failure. Biopsy revealed glomerular arteriolar thrombotic microangiopathy.

GENETICS AND PATHOGENESIS

Each of the classic Cbl group of diseases is transmitted in an autosomal recessive fashion, but Cbl X disease is X-linked recessive. In each, the activity of methionine synthase is deficient, and so is that of methylmalonyl-CoA mutase (see Figure 3.1). Methionine synthase activity has been demonstrated to be restored by the addition of methylcobalamin [34, 35]. The fundamental defect in Cbl C and D disease involves a step in cobalamin processing so early that the formations of both methylcobalamin and deoxyadenosyl cobalamin are altered. In Cbl F disease, the defect has been identified in the transport step in which cobalamin within lysosomes, once TCII is split off, is normally transported out of the lysosome to begin cofactor synthesis [28]. The transporter defect is analogous to those of sialic acid storage disease and of cystine storage disease (cystinosis) (Chapter 18).

Table 4.2 Pathological biochemistry of the urine in Cbl C and D diseases

Metabolite	Pathological (Cbl C and D)	Normal (mmol/mol creatinine)
Urinary methylmalonate	50–700	0–2
Urinary 3-hydroxypropionate	6–30	0–24
Urinary methylcitrate	30–6	0–5
Urinary homocystine	0.08–80	0–0.01

Methionine synthase activity is deficient in Cbl E and G diseases. This enzyme catalyzes the transfer of a methyl of 5-methyltetrahydrofolate to homocysteine. Mutations in the gene coding for this enzyme cause Cbl G disease. In Cbl E disease, the mutations are in the gene for methionine synthase reductase which maintains the synthase in its reduced state.

In the presence of defective cofactor synthesis, methylmalonate and homocystine accumulate. The amounts are distinctly less than in methylmalonyl CoA mutase deficiency or cystathionine synthase deficiency (Table 4.2). The diagnosis is usually made by organic analysis of the urine, which detects methylmalonate, the most abundant metabolite. Methylcitrate and 3-hydroxypropionate are also identified in this way. Screening tests for methylmalonate (Chapter 3) are also positive, and the diagnosis may first be suspected in this way. Quantitative assay of the urinary amino acids reveals elevated amounts of homocystine. It is important for this purpose to employ fresh urine.

The amounts of homocystine are not large, and this compound is unstable in urine at room temperature. Also, proteinuria may lead to binding of homocysteine, which would then be precipitated out and removed from the analysis when the urine is acidified. Screening the urine with the cyanide nitroprusside test is also positive (Chapter 18). Some patients have had hypomethioninemia and cystathioninuria [15, 19]. Homocysteine may be found in the plasma by assay for total homocysteine. Regardless of the method, homocysteine is not found in some patients with Cbl C, and even with Cbl F disease [26].

Once the biochemical diagnosis is made, complementation analysis is performed with cultured fibroblasts incubated with ^{14}C-propionate to determine the specific Cbl complementation group [36]. Studies of the uptake of ^{57}Co-cyanocobalamin by fibroblasts have indicated deficiency in the process of conversion to hydroxycobalamin and to methylcobalamin and deoxyadenoxylcobalamin in patients with Cbl C and D disease [34, 36]. Uptake was normal in other forms of homocystinuria and methylmalonic acidemia. Cells of patients with Cbl C utilize CN-Cbl poorly and cannot convert CN-Cbl to OH-Cbl [35, 37]. This could indicate a defect at cobalamin (III) reductase, catalyzing the reduction of trivalent cobalt prior to alkylation. Concentrations of B_{12} in the serum may be elevated [16].

The biochemical picture of methylmalonic acidemia and homocystinuria and the acute hematologic and clinical neurologic picture of Cbl C disease have been encountered in the exclusively breastfed infants of strict vegan mothers [30, 38], as well as in the breastfed infants of mothers with subclinical pernicious anemia and in TCII deficiency [39]. It is also seen in the Immerslund-Grasbeck defect in ileal absorption of the B_{12}-intrinsic factor complex (Figures 4.5 and 4.6) [40]. Problems in the differential diagnosis of patients with methylmalonic acidemia were highlighted by a patient who died of mutase deficiency, ultimately diagnosed when a sibling was found to have the disease, but not before the mother was incarcerated for homicide because a commercial clinical laboratory misidentified the propionic acid in the blood as ethylene glycol [41]. This experience points up the importance of quantification and identification by gas chromatography-mass spectrometry (GCMS) as opposed to identification based on elution times in gas chromatography.

The molecular nature of the disease has been explored in Cbl C since the cloning of the gene [3, 7]. The ratio of males to females approximated two [3]. The most commonly encountered mutation c.271dupA has, when homozygous, been uniformly found to lead to the early onset severe phenotype. The c.394C>T mutation has never been encountered in homozygosity among patients with this early onset phenotype. This mutation was found in 16 percent of Italian and Portuguese alleles [6] and 24

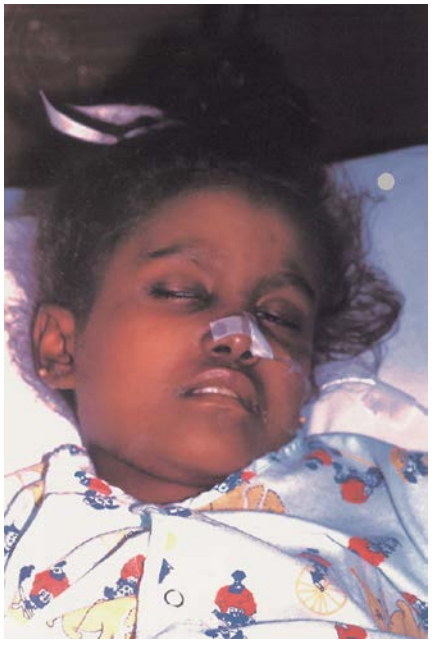

Figure 4.5 A 12-year-old girl with the Immerslund-Grasbeck B_{12} intestinal absorptive defect [40]. She was bed-ridden, semicomatose, demented, and required intragastric feeding. She had anemia for years, but developed paraparesis at ten years. The urine had increased levels of homocystine and methylmalonate. Schilling test was abnormal with and without intrinsic factor. Treatment with hydroxocobalamin led to a remarkable improvement.

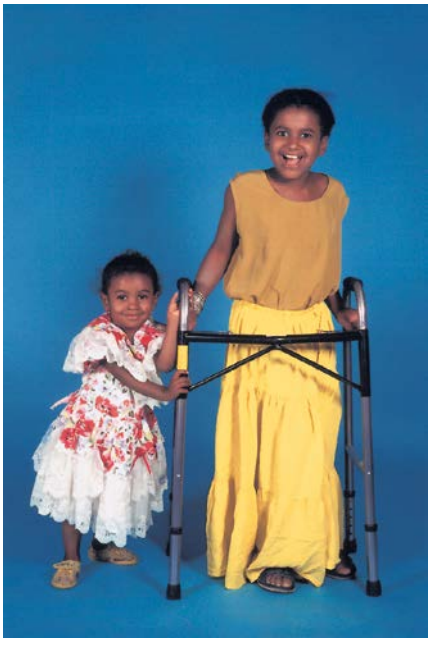

Figure 4.6 The same patient was able to walk with crutches or a walker after four months of treatment. On the left, her three-year-old sister was found to have severe anemia and was found to have the same disease. Treatment with hydroxocobalamin cured the anemia and prevented neurologic disease [41].

percent of French-Canadian alleles and essentially half of all patients [9, 10]. On the other hand, when associated with the c.271dupA mutation, the phenotype was usually early onset. A few late onset patients were compound heterozygous for c.271dupA gene and a missense mutation [10]. Late onset patients with hemolytic-uremic disease in the absence of neurologic disease were heterozygous for c.271dupA and c.82-9 12del ITTTC, an intronic mutation not otherwise encountered in the database [10]. Another late onset patient [18] had 2 mutations c.271 dupA and c.482G>A. The former is common in Cbl C deficiency. The latter appears to be a late onset allele. Two other patients with these two mutations had neuropsychiatric and hematologic, genitourinary, or skeletal problems similar to this patient. The possibility that mutation in the methylenetetrahydrofolate reductase gene (*MTHFR*) might act as a genetic modifier in Cbl C patients was explored and found not to affect age of onset, clinical phenotype, or outcome [6].

Patients with the Cbl G defect have defective conversion of cyanocobalamin to hydroxocobalamin, as seen in Cbl C. The mutation c.609+1088G>A would create a new splice acceptor site. Alternate splicing produced a transcript that encoded truncated methionine synthase, and missing exons that include the 5-methyltetrahydrofolate and Cbl binding domains.

The interaction between methionine synthase and the MMACHC product of the *Cbl C* gene appears to be involved in the regulation of cellular processing of cobalamin required for Cbl cofactor synthesis. The MMACHC protein is a cytosolic trafficking chaperone.

The gene, *MMACHC* was located on chromosome 1p34-32 [7]. It is not a member of any previously identified family of genes. Transduction of the gene into defective Cbl C fibroblasts corrected the cellular phenotype. Among 42 different mutations, many consistent with loss of function of the protein product, one, 271dupA, accounted for 40 per cent of alleles [7]. Among late onset patients 347T>C was relatively common, particularly in populations from Asia, Pakistan, India and the Middle East [7, 35]. The mutation c.271-272 was also found in four patients reported [32]. Fibroblasts of Cbl C and D do not accumulate cobalamin in lysosomes [7]. The defects have been postulated to affect a step between cellular uptake of cobalamin and the synthesis of adenosyl- and methyl-cobalamin.

The mutations identified in Cbl D affected three highly conserved amino acids p.Ala 115 Val 114 and p.Gln 68 [11]. Testing for point mutations and C-Terminal/truncation in *MMADHC* defined a region p.R197-D226 responsible for the MeCbl Cbl synthesis and a Cbl D-HC phenotype. Regions, D226 – D246 gave cellular phenotypes intermediate to Cbl D – HC and Cbl D-MMA/HC, while truncation of 10–20 amino acids gave a Cb D-HC phenotype. MMADHC does not bind cobalamin; so, an interaction with MMACHC has been proposed. The interaction would target cobalamin to the cytosolic pathway, where it could participate with methionine synthase.

The gene *HCFC1* is a coregulator of the zinc-finger-transcription factor THAP11, which is also known as RONIN. The THAP11-HCFC1 binds consensus-sequence motifs in genes encoding a number of enzymes of cobalamin metabolism, *MMACHC, MTR* and *ABCD4*. It also binds *SUCLG1* which codes for a krebs cycle enzyme, mutations in which cause markedly elevated methylmalonic aciduria. Expression levels of *HCFC1* mRNA were not different in patients and controls, indicating that the mutations do not alter its expression but rather its function in the activation of *MMACHC* RNA and protein which are markedly regulated in fibroblasts of patients. Furthermore, siRNA knock down regulated the expression of *MMACH*. The complex phenotype doubtless involves dysregulation of other targets of *HCFC1*.

Newborn screening has been proposed for Cbl C, D, and F disease based on the study of known patients [42]. In this algorithm, patients initially detected on the basis of a high C3-carnitine and C3:C2 ration would be studied for low methionine (<13.4 μmol/L) as a secondary analyte. The prevalence of Cbl C disease among the state newborn screening population approximated 1 in 100,000 live births [42].

Patients detected by newborn screening have been diagnosed definitively by complementation analysis of cultured fibroblasts, a process that might take as long as two months. Follow up by testing for the two most common genes or by adding the third c.331C>T should dramatically accelerate this process and lead to early treatment. Testing for c.271dupA has been reported to accomplish early diagnosis in two patients [6].

TREATMENT

Treatment of Cbl C disease has largely been unsatisfactory. Most patients have died or been severely handicapped. The documented poor uptake of labeled cyanoB_{12} by fibroblasts [34], indicated that these patients should be treated with hydroxocobalamin. Large doses, 1–1.5 mg intramuscularly (i.m.) each day, have been employed [43–45]. In volunteers given 5–10 g of intravenous hydroxocobalamin, dose-dependent increase in blood pressure has been lowered [45].

Among three patients with neonatal hemolytic uremic syndrome patients with Cbl C disease, treatment with parenteral hydroxocobalamin, folic acid, and betaine reduced biochemical abnormalities to normal levels [32]. There has been little consensus of the optimal dose of hydroxocobalamin beyond infancy; most infants are treated with 1 mg daily, with some reduced to less than daily use after infancy. Increasing parenteral hydroxocobalamin to 5 mg daily was required in two patients for clinical and metabolic control of thrombotic microangiopathy [46]. Others have recommended doses of 20 to 25 mg [44]. Better outcome after dose escalation has been reported [47–49]. Doses ranged from 0.11 to 0.36 mg/kg/24 hours.

Significant decreases in urinary methylmalonate have been observed and similar effects were observed in plasma homocysteine. Growth rates have become normal. Reversion to normal of abnormal visual and auditory evoked potentials has been reported [16].

Treatment with hydroxocobalamin has also been reported in Cbl E disease to resolve homocystinuria, methylmalonic aciduria, hypermethioninemia, megaloblastic anemia, and failure to thrive [48].

Supplemental uses of betaine, carnitine, and folate (folinic acid 140–350 mg/kg) have been recommended. Dietary restriction does not appear to be useful [46]. The fact that creatine synthesis from guanidinoacetate requires methyl groups provided by the conversion of methionine to homocysteine led to the finding that concentrations of guanidinoacetate are high, and those of creatine low in five patients with Cbl C disease [50]. This raises the possibility that treatment with creatine may be helpful.

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5

The methylmalonic malonic aciduria of deficiency of AcylCoA synthetase (ACSF3)

MAJOR PHENOTYPIC EXPRESSION

Organic acidemia phenotype of recurrent episodes of ketoacidosis; hypoglycemia seizures; failure to thrive; developmental delay; methylmalonic acidemia; malonic acidemia; and mutations in the *ACSF3* gene.

INTRODUCTION

The combination of malonic and methylmalonic aciduria was first described by Gregg and colleagues [1] in a child who had seizures and failure to thrive, along with immunodeficiency. Levels of methylmalonic acid in the urine were appreciably higher than those of malonic acid. Activity of malonylCoA decarboxylase was normal. Three infants reported by Ozand and colleagues [2] had severe progressive encephalopathy with dystonia, spasticity, and recurrent episodes of metabolic acidosis. In these children, the excretion of malonic acid was greater than that of methylmalonic acid; so, they may have represented a different disorder. The activity of malonylCoA decarboxylase was normal. They also excreted large amounts of lactic and pyruvic acids, citric acid cycle intermediates, and dicarboxylic acid products of disordered fully acid oxidation.

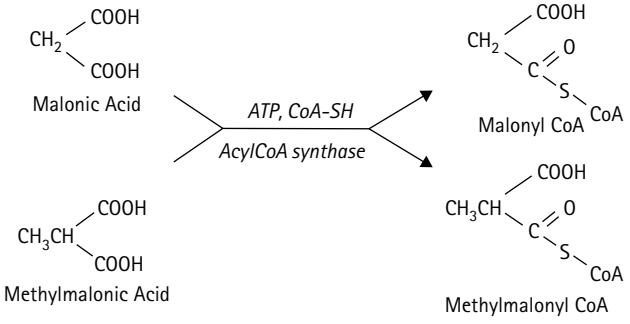

Figure 5.1 AcylCoA synthetase. Malonic acid and methylmalonic acid are substrates for the protein coded for by *ACSF3*.

We also reported [4] a dog with progressive diffuse degeneration of the brain and spinal cord. She excreted over four times as much methylmalonic acid as malonic acid. Mutations in *ACSF3* were elucidated by whole exon sequencing [3] of an index patient with ocular migraine, problems with memory and MRI evidence of T_2 hyperintensity in the brain. This was followed by candidate sequencing of eight additional patients [3]. The canine orthologue of the gene was identified, and mutational analysis revealed the dog to be homozygous for a mutant in this gene.

This was the first identification of a disease association with a member of the acylCoA synthetase (ACS) family, a group of conserved enzyme proteins involved in the activation of fatty acids to permit entry into intermediary metabolism (Figure 5.1). The enzyme activates malonate and methylmalonate to malonyl CoA and methylmalonyl CoA.

CLINICAL ABNORMALITIES

A typical organic acidemia pattern was observed in five patients, who displayed episodic ketoacidosis with or

without hypoglycemia, progressive to coma. Our patient displayed this pattern.

Its frequency and severity decreased with time. Other childhood onset patients had failure to thrive and elevated transaminases. Some had microcephaly, dystonia, axial hypotonia, and developmental delay. Four patients were diagnosed in adulthood after presenting with seizures, problems with memory, psychiatric abnormalities, or decline in cognition.

Combined malonic and methylmalonic aciduria has also been detected by newborn screening in Quebec [5]. Four patients were recognized by thin layer chromatography of the urine. All of these individuals were asymptomatic, so this was reported as a benign organic aciduria. However, most were young at the time of report. The excretion of methylmalonic acid in the urine ranged from 29.2 to 583 mmol/mol creatinine. The excretion of malonic acid ranged from 2.9 to 91 mmol/mol creatinine. Plasma levels were 5.6–48 and 1.7–11.3 μmo/L respectively. Plasma propionylcarnitine ranged from 0.19 to 0.77.

Concentrations of B_{12} and homocysteine were normal as was activity of malonyl CoA carboxylase and sequencing of its gene, and in two patients sequencing of other methylmalonic acidemia genes. Incorporation of 1-^{14}C propionate in cultured fibroblasts was normal in three patients.

The Labrador retriever presented with progressive tetraparesis. Extremity stiffness was followed shortly by ataxia. The dog became non-ambulatory and anorexic. There was marked extensor rigidity of all the limbs. Deep tendon reflexes were exaggerated, and there was crossed extension.

The excretion of methylmalonic acid was 449 mmol/mol creatinine; that of malonic acid was 101 mmol/mol.

Examination of the brain at autopsy revealed extensive atrophy of gray matter.

GENETICS AND PATHOGENESIS

In the index, patient whole exome sequencing yielded three variants in *ACSF3*. They were C.1385A>C, p.Ks 462T and c.del1394_1411, p.E 465G470del, which were in trans with c.1627C>T,p.R558W; as indicated by parental genotypes. All were confirmed by Sanger sequencing.

Sequencing of *ACSF3* exons in seven other patients yield six variations in the gene. In one patient, no mutations were found. The second patient was heterozygous for c.1567C>T and c.1672C>T,p.R523X/R558W (Figure 5.2). This latter mutation was found in two other patients, homozygous in patient four. In patient three, the other allele carried c.1075G>A,p.E359K. Patient five was heterozygous for c.1073C>T,p.T358I and c.1412G>A, pR471Q; The other three patients were homozygous for c.1411C>T,p.R471M, c.728C>T,p.P243L, and c.593T>G, p.M198R.

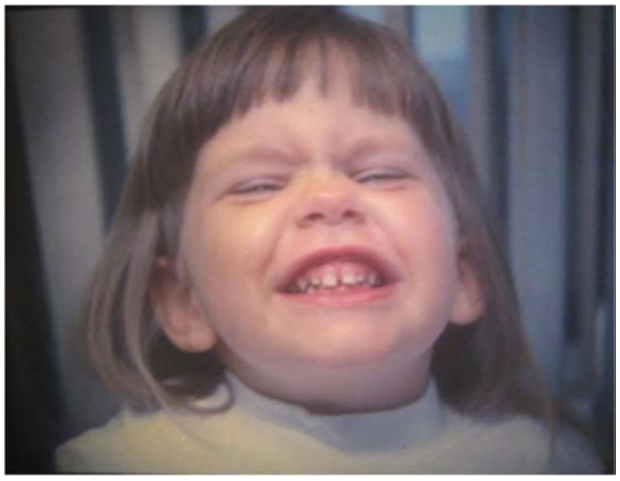

Figure 5.2 A girl with an *ACSF3* mutation, c.1672C>T (p.R858W), homozygous. In her early years, she had multiple episodes of ketoacidosis in a typical organic acidemia pattern. By the age of 18 years, she had not had an admission for this for four years. She graduated from high school and was about to start college.

The canine orthologue was sequenced in the dog, who was homozygous for c.1288G>A,p.G430S, which is orthologous to G480 in man. The variant was absent in 40 control Labrador samples of DNA.

Most of the mutations were located in the C terminal half of the gene. Eight of the nine missense mutations and the deletions were in highly conserved motifs predicted to be involved in binding of AMP or substrate, conformational change or catalytic function.

Western analysis of fibroblast extracts of five patients showed cross-reacting material. Viral expression of *ACSF3* restored function.

The *ACSF3* gene has been described as an orphan member of the acylCoA synthetase gene family. This group of enzymes catalyzes the esterification of carboxylic acid substrates to their CoA derivative. The structure of *ACSF3* was found to be similar to the malonylCoA synthase of Bradyrhizobium japonicum. Purified GST-tagged *ACSF3* activated malonate and methylmalonate to their coenzyme thioesters. The enzyme was found to be localized to the mitochondria. The gene has a mitochondrial leader sequence.

TREATMENT

In our patient, episodes of ketoacidosis were treated with intravenous (IV) fluid and electrolytes and carnitine. Thereafter, she received oral carnitine. Initially, a protein restricted diet was employed, but with time and decrease in both frequency and severity of ketoacidosis, dietary restrictions were removed.

Treatment of the Labrador retriever with carnitine, cobalamin, and a diet restricted in protein led to a marked improvement in the organic acids of the urine, but there was no clinical improvement.

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6

Multiple carboxylase deficiency/holocarboxylase synthetase deficiency

MAJOR PHENOTYPIC EXPRESSION

Erythematous, scaly eruption; alopecia; episodic, potentially lethal attacks of vomiting, ketosis, acidosis, and dehydration progressive to coma; lactic acidemia; organic aciduria including 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, methylcitric acid, and 3-hydroxypropionic acid; defective activity of the propionyl CoA, 3-methylcrotonyl-CoA and pyruvate carboxylases; and defective activity of holocarboxylase synthetase (Figure 6.1).

INTRODUCTION

The first patient described with this disorder [1] was recognized as having an abnormality of leucine metabolism by the identification of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid in the urine. When we found that methylcitric and hydroxypropionic acids were also excreted by the same patient [2], enzymatic analysis revealed defective activity of propionyl CoA carboxylases [3], as well as 3-methylcrotonyl CoA carboxylase [4]. The third mitochondrial carboxylase, pyruvate carboxylase, was also shown to be defective in activity [5]. The disorder was then renamed "multiple carboxylase deficiency" (Figure 6.2). It is now clear that there are two distinct disorders in which there is multiple carboxylase deficiency: holocarboxylase synthetase (HCS) deficiency (Figure 6.1) [6], which was the defect in the initial patient, and biotinidase deficiency (Chapter 7).

The gene for holocarboxylase synthetase has been cloned and assigned to chromosome 21q22 [7, 8]. The nature of the mutation was initially defined in two Japanese patients: a single-base deletion (1delG1067), which results in a premature termination; and a missense mutation (T997C), which changes a leucine to a proline. These mutations were

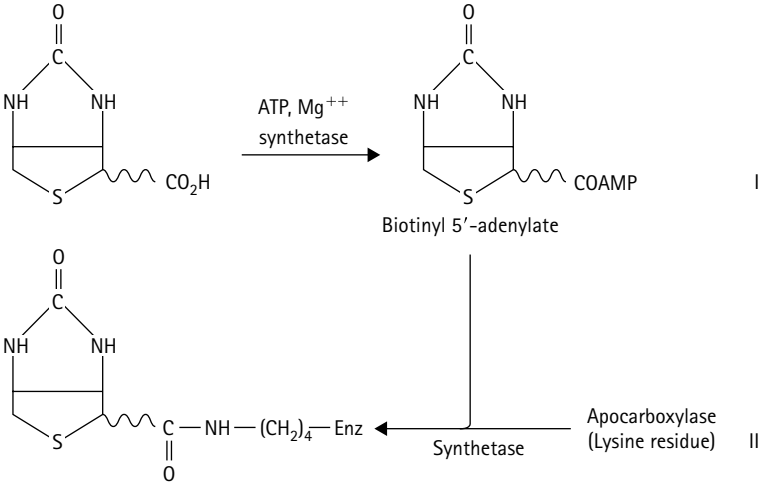

Figure 6.1 Holocarboxylase synthetase.

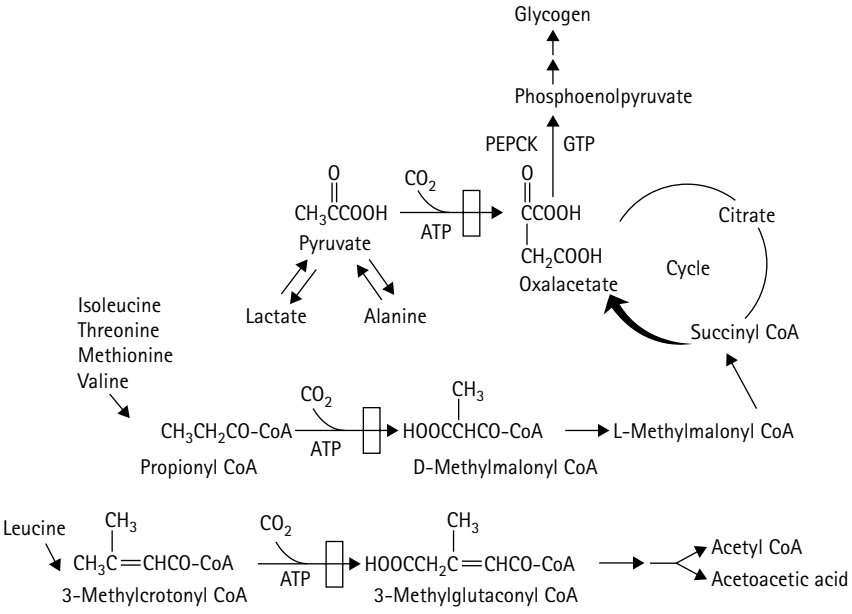

Figure 6.2 Pyruvate carboxylase, propionylCoA carboxylase, and 3-methylcrotonylCoA carboxylase. The activities of each are deficient in multiple carboxylase deficiency.

found in a number of Japanese mutant alleles. Examination of European and Middle Eastern populations has revealed a variety of mutations, none of them common [9]. Expression yielded activity ranging from 1 to 14 percent of control. Among a variety of mutations reported, p.R508W and p.V550M were found in different ethnic groups and in different haplotypes, suggesting recurrent mutation [10]. Expression of the L237P mutation yielded an enzyme with decreased activity [9].

Among the most rewarding features of the disease is the exquisite sensitivity of most of the variant enzymes to treatment with biotin, which converts an otherwise uniformly fatal disease to completely normal health.

CLINICAL ABNORMALITIES

Patients with HCS deficiency generally present in the first days or months of life with overwhelming illness identical to those of propionic acidemia (Chapter 2) or other classic organic acidemia [1, 11–16]. In seven patients in whom the enzyme defect was documented [14], the age of onset of clinical symptoms varied from the first day of life to 18 months [17]. Most patients presented before six weeks of age, but it is clear that patients with an abnormal holocarboxylase synthetase can present at any age from one day to six years of age [18, 19]. The initial impression that the two forms of multiple carboxylase deficiency could be differentiated by the age of onset has not held up, although those with holocarboxylase synthetase abnormalities [20] have generally presented within the first six weeks of life, while those with biotinidase deficiency have generally presented after six months of age.

In the acute episode of illness, the infant has massive ketosis and metabolic acidosis with an anion gap. There may be tachypnea or Kussmaul breathing. Concentrations of ammonia in the blood may be elevated. The episode may progress to dehydration, deep coma, and, unless vigorously treated, death. There is documentation of a number of patients who have died of this disease [11–21]. In fact, the initial episode may be lethal within hours of birth [11].

The classic patient with this disease was JR [1], in whom all the initial studies were performed [1–5, 22] and the defect in the HCS enzyme worked out [6, 14]. He had had recurrent episodes of vomiting from birth. An erythematous skin rash appeared at six weeks of age. At five months, he developed rapid respirations, vomiting, and unresponsiveness, and was found to have ketosis and metabolic acidosis.

The manifestations of the disease in the skin are memorable (Figure 6.3). An erythematous eruption usually involves the entire body. Some patients have died before the development of skin lesions, and now patients are being treated before the development of cutaneous lesions, but cutaneous features are an integral part of the untreated disease. The lesions are bright red, scaly, or desquamative.

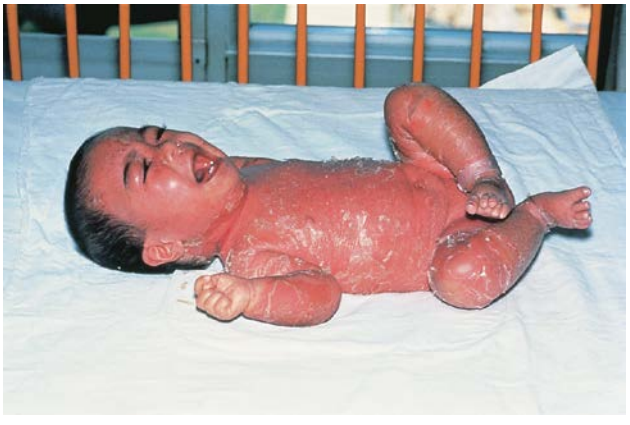

Figure 6.3 AF at nine months of age. He had a bright red scaly eruption. Alopecia was not prominent during this relapse.

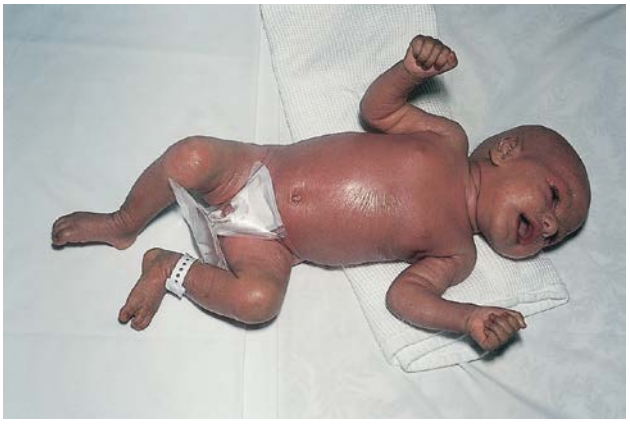

Figure 6.4 MZ at three months of age. The scaly eruption was erythematous and present throughout the body. He had almost complete alopecia of the scalp, except for a small amount of occipital hair, but there were sparse eyebrows and eyelashes.

Intertrigineous areas may be exudative. Complicating infection with monilia is common. The differential diagnosis of the skin disease includes acrodermatitis enteropathica, seborrheic dermatitis, and ichthyosis. The dermatosis is identical to that of clinical biotin deficiency [23]. Varying degrees of alopecia (Figure 6.4), an unusual manifestation in childhood, are associated, including alopecia totalis (Figure 6.4); eyelashes, eyebrows, and lanugo hair are absent, as well as the hair of the head. The differential diagnosis of alopecia includes:

- multiple carboxylase deficiency (HCS and biotinidase deficiencies)
- biotin deficiency
- cartilage hair hypoplasia
- an(hypo)hidrotic ectodermal dysplasia
- trichorhexis nodosa – argininosuccinic aciduria
- vitamin D receptor abnormalities.

Persistent vomiting may lead to failure to thrive. Neurologic abnormalities are not integral features of the disease; they appear to be related more to the effects of the initial, or repeated, episodes of illness in which there might be diminished perfusion of the brain or hyperammonemia, and the neurologic examination may be normal despite an episode of hyperammonemia [24]. Hypotonia has been observed, as well as hypertonia and irritability [25]. Athetoid movements and opisthotonus have been described [26], as has "cerebral palsy" [27]. There may be abnormalities of the electroencephalogram (EEG), and abnormalities of computed tomography (CT) or magnetic resonance imaging (MRI) scans, particularly in the white matter. An infant was reported [28] to have subependymal cysts, seen on cranial ultrasound and MRI, which disappeared following six months of treatment with biotin.

Among 22 patients with this deficiency, 86 percent had abnormal findings on imaging including ventriculomegaly and intraventricular hemorrhage [29], as well as subependymal cysts. Subependymal cysts were also observed in seven Samoan infants who had severe disease, incomplete responsiveness to biotin, and early metabolic decompensation and death [30]. Our patient with a poor dermatologic response to biotin (Figure 6.3) was Samoan; his sister died in the neonatal period [11].

Patients have disordered immunologic function of both T and B cells [11]. A diminution in the number of circulating T lymphocytes has been observed along with a diminution in their *in vitro* response to Candida in a patient with a history of bacteremia [25].

GENETICS AND PATHOGENESIS

The disorder is transmitted as an autosomal recessive trait. Both males and females are affected, and siblings of uninvolved parents have been observed. Consanguinity has been documented [1, 11]. Fibroblast cultures from two unrelated individuals were studied using the complementation assay for propionic acidemia [5]. They failed to complement each other, but they did complement mutants for all of the other groups studied, such as propionic acidemia. Heterozygote detection has not been possible by enzyme analysis, but in a family in which the mutation is known, it should not be demanding.

The metabolic hallmark of this disease is the excretion of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid along with elevated amounts of lactic acid in the blood and urine. Thus, the first clinical chemical clue to the disease may be the documentation of lactic acidemia. Organic acid analysis at the time of acute acidosis also reveals methylcitric and 3-hydroxypropionic acids. The organic acidemia may be quite variable, particularly if first studied after intensive therapy with parenteral fluid and electrolytes and resolution of the acidosis. The excretion of 3-hydroxyisovaleric acid is virtually always greater than that of 3-methylcrotonylglycine [11, 20, 26]; but occasionally, the proportion of these values was reversed [1]. The excretion of 3-hydroxyisovaleric acid may be as high as 200 times that of normal [2]. The lactic aciduria may be enormous. These patients may also excrete tiglylglycine in the urine [11].

The lactic acidosis may be striking [15]. In an infant with lactic acidosis, it is important to consider this possibility and to assay the organic acids in the urine; if organic acid analysis is not promptly available, a trial of biotin therapy is warranted (with the urine saved for analysis).

The activities of the carboxylases (Figure 6.2) may be measured in leukocyte extracts or in fibroblasts, as well as in tissues. In patients with holocarboxylase synthetase deficiency, we have found levels of activity ranging from 0.4 to 53 percent of control (Table 6.1). In parallel studies of propionyl CoA and 3-methylcrotonyl CoA carboxylases in fibroblasts, their kinetic properties were normal [3]. The levels of activity of these enzymes are dependent on the concentrations of biotin in the medium. Activity of all of the carboxylases is markedly deficient when fibroblasts are

Table 6.1 Carboxylase activity in holocarboxylase synthetase deficiency

	Normal range (pmol/min mg/protein)		Patient data (% of control)	
	Leukocyte	Fibroblast	Leukocyte	Fibroblast
Propionyl CoA carboxylase	160–447	128–537	12–43	0.7–52
3-Methylcrotonyl CoA carboxylase	62–288	71–250	15–34	0–29
Pyruvate carboxylase	7–14	96–362	29–53	2–60

grown in 6 nmol biotin/L. Carboxylases are normal when the cells are grown in 100 nmol biotin/L.

The fundamental defect is in holocarboxylase synthetase (EC 6.3.4.10) (Figure 6.1) [6, 14]. This is a complex enzyme which activates biotin to form D-biotinyl-5′-adenylate, and then catalyzes the attachment of the biotin to an ε-amino group of a lysine residue of the newly synthesized apocarboxylase enzyme. The covalent binding to biotin conveys enzymatic activity and holocarboxylase status to the apocarboxylase protein, which is inactive prior to this conversion.

Most patients studied have had altered Kms for biotin; the normal Km is 1–6 nmol/L, and values in 16 patient cells ranged from 9 to 12 nmol/L. The maximum velocity (Vmax) at saturation concentrations of biotin may be normal or reduced. There appears to be good correlation between the age of onset and severity of illness and chemical responsiveness to biotin and the degree of elevation of the Km for biotin and residual activity of holocarboxylase synthetase [10, 14]. Our patient (Figure 6.3) with 70 times normal Km for biotin presented in the first few hours of life [12], and a previous sibling had died in the neonatal period [11]. Patients in whom the Km values for biotin and residual activity of holocarboxylase synthetase were 20–45 times normal presented between one day and seven weeks of life. A patient with a Km for biotin only three times normal presented at eight months of age. The Km for biotin was not elevated in the enzyme coded for by the L237P mutation [9]; the Vmax for this enzyme was 4.3 percent of the control mean.

The initial enzymatic step, the biotinyl–AMP (adenosine monophosphate) synthetase reaction, has now been found to be deficient in each of the fibroblast lines studied [31]. In four patients studied, activity ranged from 0.3 to 8 percent of controls. This makes for a much simpler assay than that currently available for the whole reaction, in which the substrate for the synthetase is an apocarboxylase carefully purified from the liver of biotin-deficient rats. Biotinylation has also been studied by reaction with p-67, a peptide containing the last 67 amino acids of the α-subunit of propionyl-CoA carboxylase followed by electrophoretic separation [32].

The cDNA for holocarboxylase synthetase [7, 33] codes for a protein of 726 amino acids [7, 33]; amino acids 445–701 have homology with related enzymes in *E. coli* and yeast. Among the mutations identified, a number in this domain are considered to bind biotin, for instance R508W, G518E, and V550M [34]. This is consistent with the clinical biotin responsiveness of six patients.

Since the identification of the HCS cDNA sequence, 30 mutations in the gene have been reported [10]. Mutations have been identified throughout the coding region except for exon 6 and 10. Only one intronic mutation has been identified, c.151q +5G>A (IVS10+5g→a), which is a founder mutation in Scandinavian patients. The mutation is more prevalent in the Faroe Islands than in the rest of the world [10]. There has been a good relationship between clinical responsiveness to biotin and the residual activity of this enzyme.

Two mutations L237 and 1067delG have been considered founder mutations among Japanese populations [35]. Among 20 Japanese alleles, these two were found in seven and five. Five mutations accounted for 90 percent of Japanese mutant alleles.

R508W was found in three late onset Chinese patients, two of them homozygous [36]. Neonatal onset has been found in patients with null mutations and point mutations associated with very little residual activity [10].

A patient who presented unusually late (eight years of age) but had a very slow response to biotin was found to have abnormal splicing of mRNA and a modest decrease of normal mRNA, resulting from a homozygous mutation in the splice donor site (IVs 10 +5 (g→a) of intron 10 [37].

Prenatal diagnosis has been accomplished by the demonstration of biotin-responsive deficiencies of carboxylases in cultured amniocytes. It has also been demonstrated by assay of the activity of holocarboxylase synthetase [38]. Prenatal diagnosis has also been made by the direct assay of methylcitric acid in the amniotic fluid by stable isotope dilution and selected ion monitoring using gas chromatography-mass spectrometry (GCMS), as in propionic acidemia (Chapter 2). However, the concentrations of 3-hydroxyisovaleric acid in the amniotic fluid of patients with HCS deficiency are higher and its quantification is more reliable [39]. This is the best chemical method for the rapid prenatal diagnosis of this disorder and may unequivocally indicate an affected fetus. However, we have encountered the situation in which the results of this assay were equivocal, in which case, assay of holocarboxylase synthetase gave the correct diagnosis [40]. Prenatal diagnosis has been carried out by enzyme assay of amniocytes [38, 40] and chorionic villus material [41]. In each case, a markedly elevated Km for biotin was diagnostic. In a family in which the mutation is known, molecular methods may be used for prenatal diagnosis.

The L216R mutation common in Polynesians has been considered among the most severe form of HCS deficiency, and many have died in the neonatal period or early infancy

[42]. The mutation has been considered unresponsive to biotin, but in two homozygous siblings three doses of 1.2 g/day (25 mg/kg/day divided twice daily) and 40 mg/kg/day led to resolution of manifestations of disease and avoidance of further episodes of metabolic imbalance.

Among patients responding poorly to biotin, two missense mutations, L216R and L237P were shown to have reduced affinity for substrate and a more than 15-fold increase in dissociation rate [43].

Newborn screening has made an enormous difference in this disease. Patients diagnosed in this way and treated promptly do very well. Two patients with novel variants were first diagnosed at 3 years and 21 months [44]. The first had a novel newborn screen; the second was misdiagnosed as 3-methylcrotonylcarboxylase deficiency. The first patient had a known variant c.1993>T and a novel one c.500A>c. A positive newborn screening was found in the biochemically normal infant of a mother being treated for holocarboxylase synthetase deficiency [45].

TREATMENT

Virtually all patients have been exquisitely sensitive to treatment with exogenous biotin. None have had acute attacks of ketoacidosis while taking biotin. The initial dose employed was 10 mg/day, and most patients have responded well to this dose. Nevertheless, heterogeneity in this condition may be manifest in the level of responsiveness to biotin. In some patients, small amounts of metabolites, present when the dose was 10 mg of biotin per day in the urine, disappeared when it was increased to 40 mg/day [25, 38]. Another patient, although clinically well when receiving as little as 1 mg of biotin per day, had elevated excretions of metabolites and activities of the carboxylases in leukocytes that were only 4–16 percent of normal when the dose was 20 mg of biotin per day [46]. A patient with a very high Km for biotin [14] continued to have skin lesions, large excretions of metabolites and impaired activities of carboxylases in lymphocytes when receiving doses of biotin as high as 60 mg per day. Another patient required 100 mg per day before skin lesions resolved, and the relevant organic acids were present in small amounts in the urine, even on this regimen [18]. In Polynesian patients with the L216R mutation 25–50 mg/kg of biotin were required to achieve clinical responsiveness [42]. Two Polynesian patients with the L216R mutation were treated with 1.2 g of 42 oral biotin daily, the highest dose reported, hospital admissions had not been required since initiation of this dose [43].

When provided with adequate amounts of biotin, none of the patients have required dietary restriction of protein, although moderate restriction in the less responsive patients could well decrease the accumulation of metabolites.

The usual clinical response to treatment is dramatic. Ketosis and acidosis disappear along with hyperammonemia. Levels of lactic and pyruvic acid in the blood become normal [47]. Lethargy, hypotonia, and ataxia disappear [2]. The skin lesions disappear in virtually all patients and the hair grows. Abnormalities of the EEG and CT scan have been documented to disappear [12]. At the same time, persisting neurologic abnormalities, such as developmental delay [18, 26] and dilated ventricles [12] once developed would not be expected to regress. The biochemical response to treatment is striking; the levels of organic acid metabolites often decrease to normal, but it may remain possible to continue to detect 3-hydroxyisovaleric acid in the urine [20, 23]. The activities of the carboxylases in leukocytes usually become normal within a few days of the initiation of therapy.

Few pharmacokinetic data have been assembled, but blood levels of biotin approximating 100 ng/mL have been reported in a three-month-old infant receiving 10 mg of biotin per day [48]. Urinary excretion varied between 2 and 4 mg/g creatinine. In a two-year-old child receiving the same dose, plasma levels as high as 703 ng/mL were observed [26], and in a neonate a level of 660 ng/mL was found following a dose of 20 mg per day [25]. These values exceed the range of the altered Km for biotin in the patients studied and provide a potentially elegant correlation of the kinetics of each variant enzyme and the clinical pharmacology of biotin. A patient who responded nicely to 100 mg of biotin daily after a poor response to 10 mg/day achieved plasma concentrations of over 500 nM, demonstrably higher than the Km of the enzyme for biotin of 164 nM [32]. Measurement of these pharmacokinetic data provides an elegant approach to the determinations of optimal treatment.

A patient who developed seizures at six months and periorificial dermatitis at four years of age was found to have normal biotinidase activity, and the typical organic aciduria was diagnosed as having holocarboxylase synthetase deficiency [2]. Treatment with 5 mg of biotin daily led to resolutions of skin lesions and cessation of polydipsia and polyphagia as well as normalization of weight.

Prenatal therapy with biotin has been successfully pursued in at least four pregnancies at risk [38–41, 49]. In most, the affected fetus was diagnosed prenatally [38–40]. The dose of biotin to the mother was 10 mg/day, and levels of biotin in maternal serum were very high. There were no ill effects in the mother or fetus. At birth, assay of holocarboxylase synthetase in cultured skin fibroblasts indicated that infants were affected, but they were clinically well and had levels of urinary organic acids that were normal (Figure 6.5). Prenatal treatment in this condition appears prudent, because birth itself may be sufficiently catabolic that an affected infant may become irreversibly moribund within hours of birth [11]; and certainly, death following ketoacidosis and disseminated intravascular coagulation has been recorded even after initiation of biotin therapy [50]. An infant, in whom treatment was carried out prenatally without diagnosis, then suspended while fibroblast cultures were established and an enzymatic diagnosis made, developed severe ketoacidosis, lactic acidemia and shock, but did respond to biotin therapy [49].

Figure 6.5 Prenatal diagnosis of holocarboxylase synthetase. JW, the boy on the left, presented with typical neonatal ketoacidosis, but was diagnosed promptly and treated with biotin and has not had a further episode. His sister, on the right, was diagnosed and treated prenatally and has never had symptoms of multiple carboxylase deficiency. The brother in the middle was normal.

Measurement of pharmacokinetic features was reported [51] to lead to optimal treatment and tailoring of dose of biotin to the mutation. In a patient with increased Km and a normal Vmax and compound heterozygosity for Q379X and Y663H, there was poor incorporation of biotin into carboxylases and transfer to the holocarboxylase synthetase enzyme. Treatment with 100 mg per day improved biochemical data in blood and CSF, clinical stability and normal neurodevelopment.

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7

Multiple carboxylase deficiency/biotinidase deficiency

MAJOR PHENOTYPIC EXPRESSION

Seizures, ataxia, hypotonia, alopecia, periorificial cutaneous eruption, hearing loss, loss of vision, myelopathy, developmental delay; episodic metabolic acidosis, lactic acidemia, propionic acidemia, excretion of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, methylcitric acid, and 3-hydroxypropionic acid in urine; and defective activity of biotinidase.

INTRODUCTION

Biotinidase deficiency is a form of multiple carboxylase deficiency in which the fundamental defect is an inability to cleave biocytin (Figures 7.1 and 7.2), and this leads to defective activity of propionylCoA carboxylase, 3-methylcrotonylCoA carboxylase, and pyruvate carboxylase [1]. Multiple carboxylase deficiency is also caused by defective activity of holocarboxylase synthetase (Chapter 5) [2]. In earlier literature, biotinidase deficiency was referred to as the later infantile form of multiple carboxylase deficiency [1, 3] to distinguish it from the usual neonatal presentation of holocarboxylase synthetase deficiency. However, it is now clear that the latter disorder can present later and the former

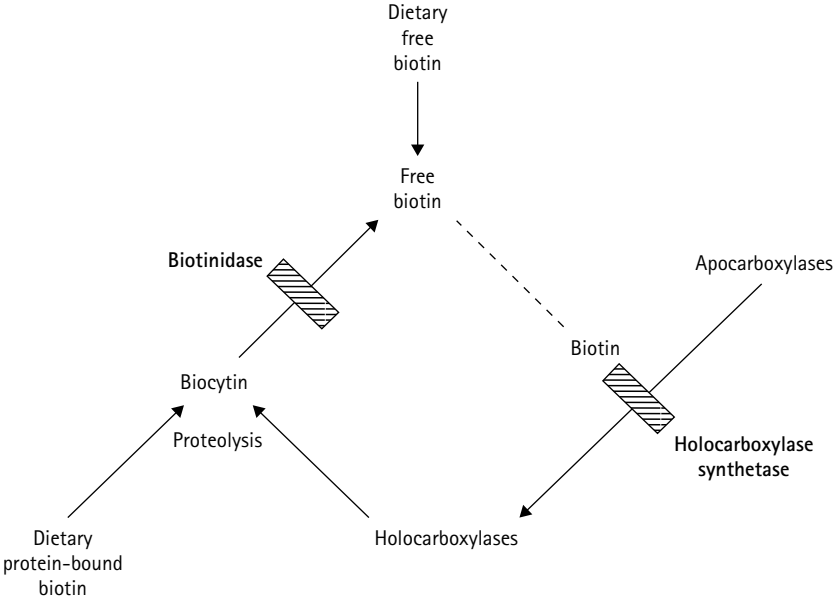

Figure 7.1 Pathways of metabolism of biotin. Biotin is an essential cofactor for all carboxylase enzymes. Attachment to the inactive newly synthesized apocarboxylase is catalyzed by holocarboxylase synthetase. Biotin is recycled through the activity of biotinidase, and this enzyme would also be required to release biotin bound to protein in the intestine.

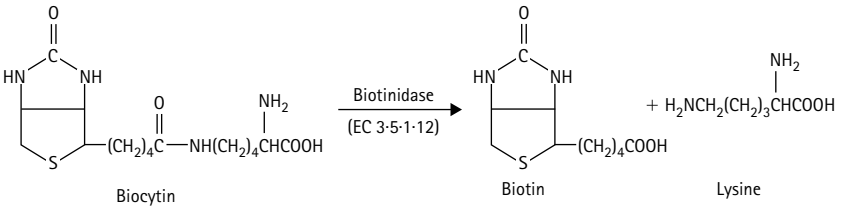

Figure 7.2 The biotinidase reaction in which biocytin is cleaved to generate biotin and lysine.

earlier; the way to distinguish them unambiguously is by enzyme analysis or determination of mutation.

Biotin, as a vitamin, cannot be synthesized by humans, but in addition to dietary sources, it is synthesized by intestinal microflora. There are dietary sources of free biotin, but covalently bound biotin must ultimately be acted upon by biotinidase to make biotin available from either dietary, intestinal bacterial or recycled sources (Figures 7.1 and 7.2). Biotin is an intrinsic cofactor for each of the carboxylase enzymes, which are synthesized as inactive apoenzymes and must be linked with biotin in the holocarboxylase synthetase reaction (Chapter 5) to become active holoenzymes.

The cDNA for biotinidase has been cloned [4], and the gene has been mapped to chromosome 3p25 [5]. At least 21 mutations were found [6] in 37 children with profound, symptomatic deficiency of biotin. Two were common, accounting for over half of the alleles studied, one a deletion/insertion delG98-G104: insTCC (G98:del7ins3) which results in a frameshift and premature termination and the other R538C, a substitution of T for C at nucleotide 1612, only five amino acids from the carboxy terminus of the enzyme. Many variants with partial deficiency of biotinidase have the mutation D444H, in compound with a mutation causing profound deficiency [7, 8]. More than 165 mutations have been reported [8]. The advent of newborn screening has identified three more novel mutations that lead to mild or partial deficiency, even when combined with a mutation such as R538C [8].

Most of the clinical abnormalities of biotinidase deficiency respond well to relatively small doses of biotin. However, optic atrophy, hearing loss, and spastic diplegia once developed do not usually resolve. In a series of 20 Turkish patients [9], all of those with hearing loss had null mutations, but three children ascertained and treated early because of an affected sibling, had normal hearing despite null mutation. This provides further argument for newborn screening. It also provides argument for mutational analysis in the counseling of families.

CLINICAL ABNORMALITIES

Biotinidase deficiency presents with a median age of three months or as late as ten years of age. Symptoms may begin in the neonatal period. Initial symptoms may be dermatologic [10] or seizures [11]. Early infantile seizures may be myoclonic.

The cutaneous lesions tend to be patchy [11–15] (Figures 7.3–7.11), in contrast to the total body eruption

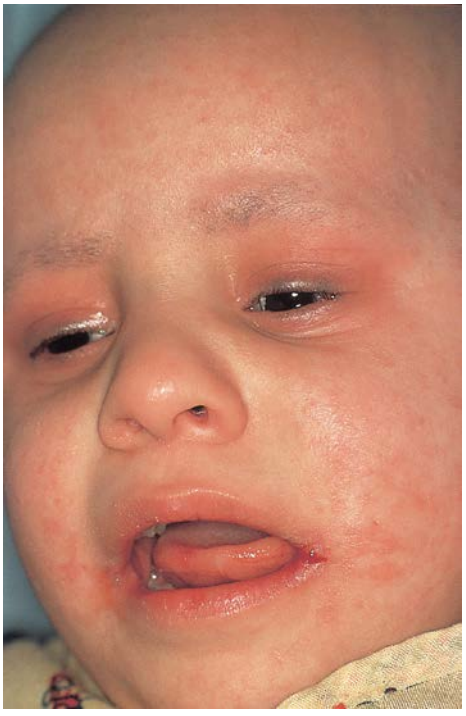

Figure 7.3 FE: A 27-month-old Saudi Arabian girl with biotinidase deficiency. She lost all scalp hair and eyebrows at 20 days, but it returned; scalp hair disappeared again at eight months, an indication of failed compliance. In addition, she had reddened dermatitis about the eyes and mouth, with cracking at the corners of the lips.

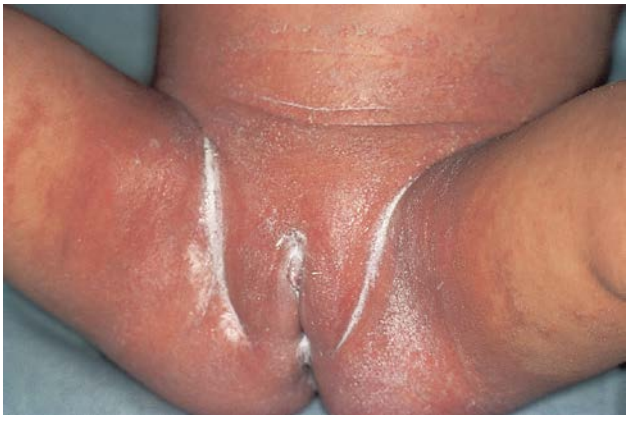

Figure 7.4 FE: There was also extensive perineal dermatitis. In addition, she had spastic quadriparesis.

Figure 7.5 FE: After six months of treatment with biotin, she had lost the spastic quadriparesis, an exception to the usual rule, had lost the dermatitis and had abundant hair.

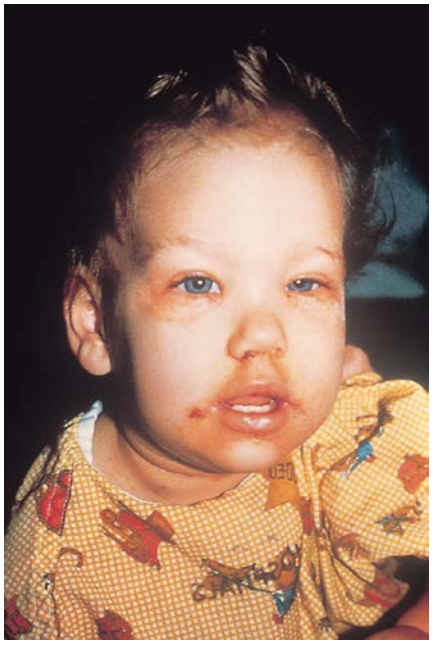

Figure 7.7 RR: A girl with biotinidase deficiency, illustrating the periorificial lesions that led to a diagnosis of acrodermatitis enteropathica. The hair was sparse. (Illustration was kindly provided by Dr Seymour Packman, University of California, San Francisco.)

Figure 7.6 FE: Six months later, illustrating the return of the alopecia following noncompliance with biotin therapy. She again responded to treatment, but essentially, had total loss of hearing.

Figure 7.8 RR: Illustrating the response to treatment with biotin. (Illustration was kindly provided by Dr Seymour Packman, University of California, San Francisco.)

seen in holocarboxylase synthetase deficiency (Chapter 5). However, there may be severe generalized involvement of the skin with redness and desquamation [13]. Skin lesions are associated with periorificial cracking, and there may be blepharoconjunctivitis or keratoconjunctivitis of sufficient severity to lead to admission to hospital. Perioral stomatitis is regularly seen, and there may be glossitis. There may also be perineal dermatitis (Figure 7.4) [13]. One of our patients had carried a clinical diagnosis of acrodermatitis enteropathica for many years. Anhidrotic ectodermal

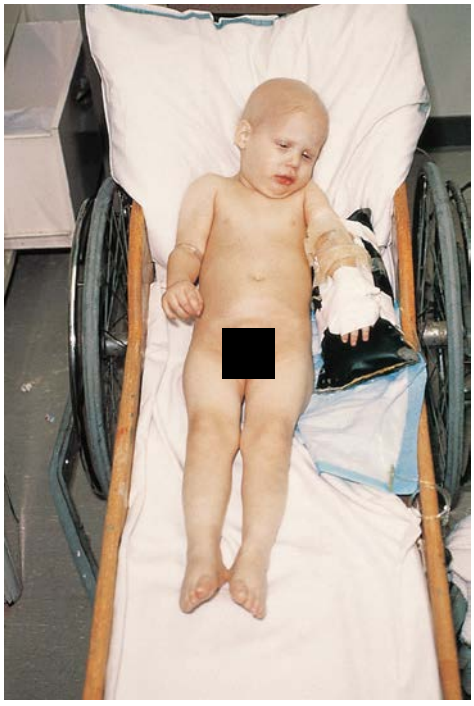

Figure 7.9 JRo: A patient with biotinidase deficiency. In addition to the skin lesions and the alopecia, incapacitating neurologic disease made her bedridden. (Illustration was kindly provided by Dr Jess Thoene.)

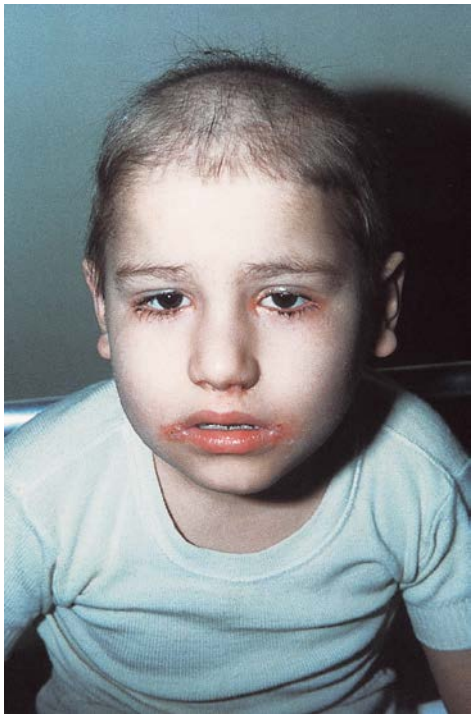

Figure 7.10 CG: A boy with biotinidase deficiency, illustrating the characteristic lesions about the mouth and eyes and the alopecia. (Illustration kindly provided by Dr E Zammarchi, Dipartimento di Pediatria, Clinica Pediatrica 1, University of Florence, Florence, Italy.)

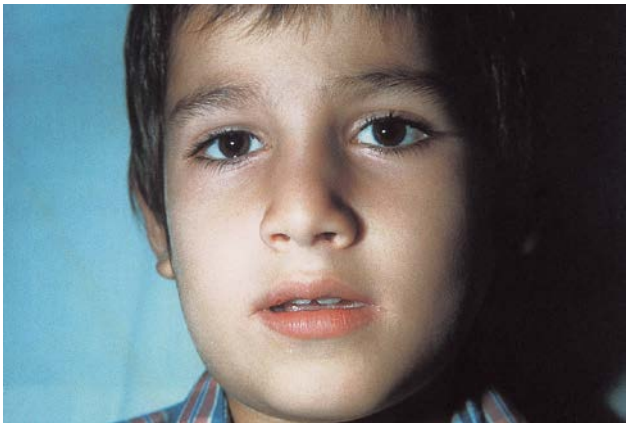

Figure 7.11 CG: Illustrating the complete reversal of the cutaneous lesions and the alopecia after treatment with biotin. He had significant hearing loss and pale optic discs. (Illustration kindly provided by Dr E Zammarchi, Dipartimento di Pediatria, Clinica Pediatrica 1, University of Florence, Florence, Italy.)

dysplasia has also been considered in the differential diagnosis of this disorder. The eruption may appear seborrheic. Mucocutaneous candidiasis is a frequent concomitant. The alopecia may be progressive to alopecia totalis (Figure 7.10) [11], but it is usually less than total (Figures 7.7 and 7.11), and may be simply a sparseness of cranial hair, eyebrows, or lashes.

In initial experience, the diagnosis was made in each patient because of the occurrence of typical episodes of acidosis, ketosis, and organic aciduria [3]. Severe, life-threatening acidosis may be seen [14], along with coma, hypothermia, massive hypotonia, and absent reflexes [16]. There may be chronic compensated acidosis with serum concentrations of bicarbonate in the range of 15 mEq/L [11]. Episodic acidosis may be seen at times of acute infection [12].

Neurologic manifestations are major features of biotinidase deficiency. Ataxia is a prominent feature and may be so profound as to interfere with walking [11]. Ataxia may also be intermittent [15]. There may be associated intention tremor. Seizures occur in over 70 percent of patients and may be the only obvious symptom [17, 18]; so, testing for biotinidase deficiency is warranted in any patient with unexplained seizures. Seizures may be generalized or myoclonic. They may be frequent, or intermittent, or they may occur only with fever. Infantile spasms may be the initial presenting feature of the disease [19]. Experience with this patient emphasized that an early neurologic presentation may lack any of the characteristic findings in the skin and hair. In one study [18] of 78 children, 55 percent had seizures, and of these, seizures were the presenting complaint in 70 percent. Seizures were poorly controlled with anticonvulsant medication in 40 percent, but in 75 percent they disappeared after treatment with biotin. This experience, like responses to biotin in holocarboxylase

deficiency, can be among the most striking and rewarding in medicine. Development may be delayed [11, 13]. Hypotonia has also been observed in over half of the patients [3]. Two patients developed acute severe hypotonia at ten months in which there was loss of head control [20, 21]. The neurologic degenerative picture of Leigh syndrome has been described in a number of patients [13, 22]. Spastic para-or tetraplegia has been reported [23] with or without loss of vision. This picture is seen mostly in older children with delayed onset, but has also been seen in younger children.

Stridorous or labored breathing and apnea have been seen in these patients [24], followed by psychomotor regression or bulbar symptoms [23]. Laryngeal stridor, a presenting feature in some patients [24, 25] has been interpreted as neurologic in origin, and it has resolved with treatment with biotin [25]. Deep tendon reflexes may be brisk. Death from the disease has been reported at nine months and at three years of age [15]. Elevated concentrations of lactate have been found in cerebrospinal fluid of a number of individuals [23].

Neurosensory abnormalities involving the optic and auditory nerves have been observed in a considerable number of patients, often as late manifestations [3, 26–31]. Loss of visual function is associated with optic atrophy [3, 26, 30]. It appears to be more common in patients in whom diagnosis and treatment is delayed [32]. Neurosensory hearing loss seems to follow the same pattern [3, 27]. Among 33 children diagnosed because of symptoms, and none treated from birth, 76 percent had hearing loss [33]. A patient diagnosed at ten months of age and treated with 10 mg of biotin per day [20, 28] subsequently developed sensorineural hearing loss and severe myopia with progressive retinal epithelial dysplasia and optic atrophy. Treatment with biotin does not necessarily prevent these optic and auditory manifestations, but the authors of the report [33] were not aware of hearing loss in any infants diagnosed via newborn screening and treated from the neonatal period. Many of the neurologic features of disease disappear in response to treatment with biotin, as do the cutaneous and metabolic features; but sensorineural abnormalities involving the optic and auditory nerves are persistent. Since most of these patients have been treated for some time before these lesions are detected, the question has been raised that they might be a consequence of treatment with biotin; however, this seems unlikely because these complications have never been encountered in patients with holocarboxylase synthetase deficiency who have been treated with biotin from an earlier age, sometimes with higher doses. Furthermore, these abnormalities have been observed in a number of patients prior to the initiation of treatment with biotin [28, 33, 34]. One patient, who presented and was diagnosed at five years of age, had already developed sensorineural abnormalities of the optic and auditory nerves, which did not resolve with treatment [33].

An unusual late-onset presentation has been described [29, 35–37] in patients with spastic paraparesis studied at 13 and 15 years of age (Figure 7.12). In each, there was progressive optic atrophy. In one boy, the first symptom was acute loss of vision during an intercurrent infection [33]. In this patient, there was improvement in visual acuity and disappearance of pyramidal tract signs in the lower limbs after months of treatment with biotin. In the other [29], there was considerable improvement in both areas, but he had spasticity and was essentially wheelchair-bound. Acute loss of vision, optic atrophy, and spastic paraparesis developed in one patient at ten years of age [36]. In the Turkish series of patients [9] with profound deficiency of biotinidase, 55 percent had hearing loss.

Figure 7.12 FM: An 18-year-old man with biotinidase deficiency who presented at 13 years with spastic diplegia and loss of vision. Vision improved with biotin, but optic discs were white and he remained wheelchair-dependent.

Immunodeficiency has been reported [14] and there have been abnormalities in the function of both T and B cells. In two patients with extensive chronic mucocutaneous candidiasis, responses to Candida antigen *in vitro* and *in vivo* were absent. In one, there was a deficiency of IgA and no antibody response to immunization with pneumococcal polysaccharide; in the other, the percentage of circulating T lymphocytes was abnormally low. In this family, two previous siblings had died at eight and 39 months of age of what appeared to be the same disease [14, 38]. An unrelated patient [39] was reported to have impaired lymphocyte-suppressing activity *in vitro* that improved on treatment with biotin and fatty acids. In this patient, there was deficiency of prostaglandin E, as well as defective monocyte production. Deficiency of biotin in guinea pigs has been associated with decreased numbers of T and B lymphocytes [40]. All of these immunologic problems disappear with biotin treatment. One patient was initially thought to have

severe combined immunodeficiency and treated with bone marrow transplantation, but manifestations persisted until treatment with biotin was initiated [41].

The electroencephalograph (EEG) is often normal in biotinidase deficiency, but among children with seizures there were 16 abnormal EEGs [18]. Diffuse slowing or convulsive activity has been observed, and usually the EEG has rapidly become normal with biotin treatment [42]. Visual evoked potentials (responses) (VEP, VER) have been abnormal in a number of patients [43, 44], returning promptly to normal with biotin treatment. In a 13-year-old boy with optic atrophy and spastic paraparesis [29], positron emission tomography (PET) showed a low relative metabolic rate for glucose in temporal and occipital lobes, which became normal following treatment.

Neuroimaging studies have been highly variable. Calcification of the basal ganglia was reported in a 26-month-old infant [45]. Magnetic resonance imaging (MRI) and computed tomography (CT) evidence of white matter lucency indicative of delayed myelination and diffuse atrophy have been reported [46, 47]. Imaging of the spinal cord has revealed hypodensity, demyelination and/or edema of the spinal cord. [23].

The neuropathology has been characterized by atrophy, and there has been neuronal loss in the cerebellum [14]. Atrophy of the superior vermis has been associated with virtually complete disappearance of the layer of Purkinje cells. In addition, there was moderate gliosis in the white matter and a subacute necrotizing myelopathy. The histopathologic picture of subacute necrotizing encephalopathy has been reported in a patient with the clinical picture of Leigh syndrome [23]. There was rarefaction and spongy degeneration in the subcortical white matter, the midbrain, pons, and medulla.

The advent of newborn screening for biotinidase deficiency has raised the possibility that some untreated patients may be asymptomatic. Some with partial deficiency have been asymptomatic until stressed by significant infection [8]. On the other hand, unrelated asymptomatic adults have been diagnosed because their children failed newborn screening [48].

Newborn screening identifies infants with profound deficiency of biotinidase, defined as activity less than 10 percent of control mean; it also identifies those with partial deficiency (10–30 percent of control). Most, but not all, of those in the partial group have not developed clinical manifestations of disease.

GENETICS AND PATHOGENESIS

Biotinidase deficiency is transmitted in an autosomal recessive pattern. Siblings of uninvolved parents have been observed and consanguinity has been documented [16, 21]. Parents of patients display about 50 percent of normal activity of biotinidase consistent with heterozygosity [49]. Biotinidase activity is detectable in normal amniocytes, so that prenatal diagnosis of biotinidase deficiency should be possible, but this has not yet been reported, although prenatal assay of the enzyme in amniocytes and chorionic villi has yielded evidence of normal fetuses and a heterozygote [50, 51]. In a family in which the mutation is known, molecular analysis is the method of choice for prenatal diagnosis and carrier detection.

Metabolic abnormalities in biotinidase deficiency include lactic acidemia, in the presence or absence of recurrent episodes of ketoacidosis. For this reason, testing for biotinidase deficiency is of interest in any patient with unexplained lactic acidemia. In young infants, there may be hyperammonemia during acute episodes of illness. The characteristic organic aciduria consists of the excretion of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, 3-hydroxypropionic acid, and 2-methylcitric acid [3, 10]. Some patients with otherwise typical phenotypes and enzyme deficiency have been reported not to have organic aciduria [3, 52, 53]. Elevated concentrations of lactate and pyruvate have been reported in the cerebrospinal fluid (CSF) [54], as was reversion to normal with treatment. In fact, an elevated CSF concentration of lactate may occur in the absence of hyperlactic acidemia [23, 42, 54, 55]. The CSF to plasma ratio of lactic acid may be as high as 3.7 [55]. The concentration of 3-hydroxyisovaleric acid may also be higher in CSF than in plasma [55].

The diagnosis is made by the assay of biotinidase (see Figure 7.2) in serum [1, 49]. The enzyme has also been shown to be deficient in the liver. Methods available for biotinidase include the cleavage of N-biotinyl-3-aminobenzoate [49], of biotinyl-^{14}C-*p*-aminobenzoic acid [56] or of the ^{14}C-labeled natural substrate N-biotinyllysine [57] and a sensitive fluorimetric method with biotinyl-6-aminoquinoline [58], which has advantages for kinetic and other studies over the release of *p*-aminobenzoate followed by diazotization in the Bratton–Marshall reaction. Values obtained with the various assays have been quite similar. In the original assay [3, 49], the mean was 5.80 and the range 4.30–7.54 nmol/min/mL, whereas values obtained in patients ranged from undetectable to 0.18 nmol/min/mL. Small amounts of biotinidase activity are detectable in normal fibroblasts, while none was found in patient fibroblasts.

Wolf and colleagues [59] have developed a simple colorimetric test for biotinidase deficiency that can be employed with spots of blood dried on filter paper, and they have demonstrated that the test is suitable for incorporation into a statewide program of neonatal screening. Two infants with the disease were identified among the first 81,243 infants screened. This led to the discovery of two more patients who were previously undiagnosed siblings. By now millions of newborns have been screened throughout the world and the yield of known patients has resulted in an estimate of frequency of biotinidase deficiency of one in 60,000 births [60].

Patients have been classified as profoundly or partially deficient on the basis of phenotype, the presence or absence of immunoreactive enzyme – cross-reacting material

(CRM) – and the isoform pattern of sodium dodecylsulfate (SDS) immunoblots [61]. Most patients were CRM-positive and had normal kinetics of the enzyme. In one variant patient with late onset disease [36], the plasma biotinidase enzyme displayed biphasic kinetics with two different low values for Vmax and two Km values. Among patients with profound deficiency of biotinidase, no relationship could be discerned between either age at onset or severity of symptoms and the CRM status or isoform patterns [61]. On the other hand, it is clear that there is a population of patients with partial deficiency identified by newborn screening who are biochemically different from those who have come to attention because of symptomatic disease.

The cloning of the gene for biotinidase and the identification of the nature of mutation has brought better correlation of phenotype with genotype. The relatively common R538C mutation involves a CpG dinucleotide, a likely place for mutation [6], and this and the G98:d7i3 cause severe disease. On the other hand, compounds of the D444H with a variety of mutations including T404I and C594delC are associated with partial deficiency [62, 63]. In a series of 34 patients, four common mutations represented 78 percent of alleles, and the D444H mutation was found in 12 of 13 with a mild disease [64] usually with a mutation causative of severe disease on the other allele. Patients with late-onset disease, spastic paresis and optic problems had mutations L215F, R538C, V457M, and G98:d7i3, which are usually found in infantile-onset severe disease [37]. The T171G mutation has been observed only in Turkish populations [65]. In this population, many patients are homozygous for null mutations such as this one [9], and all of those with null mutations had hearing loss, except for those treated early because of an affected sibling. On the other hand, all six children homozygous for missense mutations had normal hearing. The authors pointed out the advantages of homozygosity for making genotype phenotype correlation. Correlation of mutation with enzyme activity was less good, and they attributed the detectable activity in patients with null mutations to variability in measuring activity of the enzyme.

Among eight novel mutations identified by newborn screening [8], a unique mutation in an intron led to altered expression of biotinidase mRNA which led, in turn, to deficiency of enzyme activity. This mutation c.310-15delT was the first intronic mutation discovered in the gene. An online data base of biotinidase mutations has been complied [66].

The immediate consequence of biotinidase deficiency is that levels of biotin in blood, urine, and tissues are low [11]. The low level may be more dramatically evident in the urine than in the blood. These observations initially suggested a defect in the absorption or transport of biotin [67] or its excessive renal excretion.

Biocytin has been detected in the urine of patients with biotinidase deficiency [68]. This compound is not detectable in normal urine. The levels of biocytin were considerably higher than the levels of biotin when the patients were not being treated with biotin. If the normal renal clearance of biotin at half that of creatinine is a reflection of renal reabsorption, the increased clearance of biotin in biotinidase deficiency might be caused by an inhibition of biotin reabsorption by the elevated amounts of biocytin. Biocytin could compete with biotin as substrate for holocarboxylase synthetase, increasing the concentration of biotin needed for effective holocarboxylase synthesis. It is also possible that elevated levels of biocytin are directly toxic, but there is no evidence for this, as yet. A specific high-performance liquid chromatography (HPLC) method for biocytin has been developed [69]. In patients, prior to treatment, levels of biocytin in urine ranged from 7.2 to 28.8 nmol/mmol creatinine. During therapy, levels increased 1.3- to 4-fold, but increase in dosage to 200 mg/day in a patient did not change the excretion of biocytin from the level observed with 10 mg/day. Other derivatives of biotin were found in the urine and tentatively identified as bis-norbiotin and oxidation products.

The low tissue stores of biotin that result from biotinidase deficiency lead to deficient activity of carboxylases (see Figure 7.2), and this of course results in the lactic acidemia and the accumulation of the other organic acid metabolites. Activities of carboxylases were found to be more severely compromised in the brain than in the liver and kidney [23], which would be consistent with the higher levels of lactate and 3-hydroxyisovalerate found in CSF.

Activities of carboxylases in freshly isolated lymphocytes are low. On the other hand, activities of each of the carboxylases in cultured fibroblasts are normal whether cells are cultivated in high or low concentrations of biotin [12, 70–72]. This is typical of biotinidase deficiency and was used to distinguish these patients from those with holocarboxylase synthetase deficiency in whom fibroblasts display lower activity of carboxylases when grown in media containing low concentrations of biotin [73]. A rapid diagnostic method for distinguishing holocarboxylase synthetase abnormality from biotinidase deficiency is the assay of the activity of carboxylases in freshly isolated lymphocytes in the presence and absence of preincubation with biotin [72]. Of course, direct assay of the relevant enzyme is diagnostic. Activity of holocarboxylase synthetase is normal in patients with biotinidase deficiency.

Biotinidase in human and rat brain is much lower than in other tissues, and biotin levels in the brain are depleted in biotinidase deficiency earlier and more severely than in other tissues [23, 53]. This inefficient recycling of biotin would make the brain more dependent on transfer of biotin than other tissues, and thus more susceptible to deficiency. This would be consistent with the preferential elevation of lactate and 3-hydroxyisovalerate in the CSF and the occasional absence of organic aciduria, as well as the concomitance of CSF accumulation in patients with neurologic symptoms. Pyruvate carboxylase may be predominantly affected by biotinidase deficiency in the brain. Neurologic symptomatology could be the result of the toxic effect of local accumulation of lactate and organic acids. These

observations are consistent with the fact that sometimes abnormalities of the central nervous system (CNS) are the first manifestations of the disease. It is of interest that high CSF concentrations of lactate and 3-hydroxyisovalerate are not seen in holocarboxylase synthetase deficiency, where CNS abnormalities are not expected, except as a result of a catastrophic event. Concentrations of 3-hydroxyisovalerate in the CSF were high in a patient with isolated deficiency of 3-methylcrotonyl CoA carboxylase, and this patient had severe neurologic abnormalities, indicating that this compound, as well as lactate, may be toxic to brain.

TREATMENT

Patients are effectively treated with relatively small doses of biotin. The dose most commonly employed is 10 mg/day, but as little as 5 mg/day has been effective [12]. The organic aciduria and virtually all of the clinical manifestations of the disease disappear promptly after the initiation of treatment. However, auditory and optic nerve losses are not reversed [11, 14, 18, 27, 37, 74, 75]. Presymptomatic treatment in a patient diagnosed by assay of cord blood, because the disease had previously been diagnosed in a sibling, has been followed by completely normal development, including vision and hearing, for 14 months at report [76]. In one patient, oral and cutaneous administration of unsaturated fatty acids was followed by remission of alopecia and cutaneous lesions [77], suggesting that a deficiency of acetyl CoA carboxylase required for fatty acid synthesis is involved in the pathogenesis of these manifestations.

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8

Isovaleric acidemia

MAJOR PHENOTYPIC EXPRESSION

Episodic overwhelming illness with vomiting, ketosis, acidosis, and coma in ~60% of patients, chronic intermittent disease with episodes of metabolic acidosis and psychomotor retardation in ~40%; characteristic odor; urinary excretion of isovalerylglycine and 3-hydroxyisovaleric acid; C5 and C5/C3 acylcarnitine profile; deficiency of isovaleryl CoA dehydrogenase; and mutations in the IVD gene on chromosome 15q14-q15.

INTRODUCTION

Isovaleric acidemia was the organic acid disorder first described in 1966 by Tanaka and colleagues [1, 2]. It was the unusual odor that led to the recognition of the disorder as an inborn error of metabolism [1, 2]. The smell, that of typical volatile, short-chain organic acid, was so recognized by two chemists, LB Sjostrim and D Tokendall, in the original patients. It was then identified as isovaleric acid by gas chromatography. The molecular defect is in the enzyme isovaleryl CoA dehydrogenase (Figure 8.1) [3]. The corresponding gene *IVD* containing 12 exons has been localized to chromosome 15q14-15 [4, 5]. Mutations have been reported, including missense point mutations, deletions, an in-frame mutation, and mutations that result in novel processing of this mitochondrial enzyme, such as a variant that causes a mRNA splicing error deleting exon 2 and producing a truncated protein that fails to interact properly with receptors for import into mitochondria [6–10]. The advent of expanded programs of newborn screening suggests an incidence of ~1:80.000 newborns [11] but has uncovered individuals with a likely asymptomatic course. A single mutation C932T leading to an A282V protein was found in 47 percent of mutant alleles in subjects whose condition was detected through newborn screening [10, 11]. The urinary isovalerylglycine excretion of these patients was lower than those of patients identified because they became symptomatic, as were the C5 carnitine levels in the newborn blood spots despite the fact that enzyme activity was zero. By

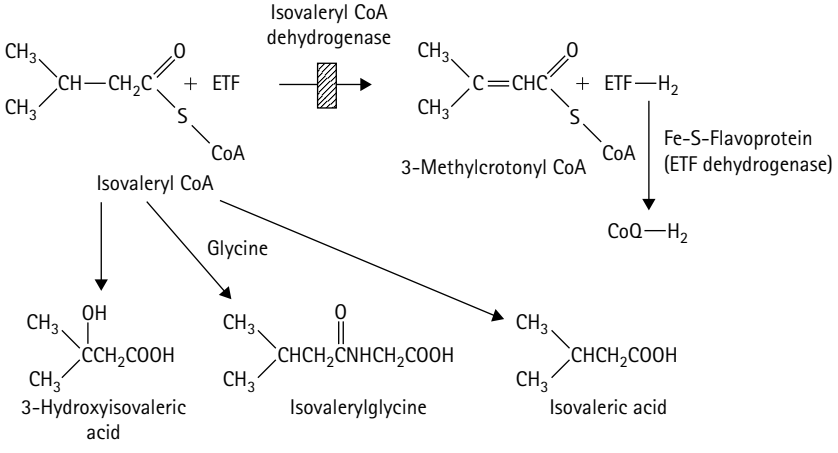

Figure 8.1 Isovaleryl CoA dehydrogenase, the site of the molecular defect in isovaleric acidemia. The characteristic urinary metabolites in this disease are isovalerylglycine and 3-hydroxyisovaleric acid.

the time of report, up to 15 years of age, none had become symptomatic. Furthermore, six older siblings were identified to have the same genotype, and none of them had ever been symptomatic. In countries where isovaleric acidemia is not part of population newborn screening, the diagnosis can be missed. The cases reported so far do not seem to cluster in certain ethnic groups. Selective screening in Oman, Thailand, and Hong Kong demonstrated the presence of isovaleric acidemia in different populations around the world [12–15].

CLINICAL ABNORMALITIES

About 60 percent of patients with isovaleric acidemia present with an organic acidemia picture of acute overwhelming illness in the first days or weeks of life (Figures 8.2 and 8.3) [16–22]. The onset is usually with vomiting, hyperexcitability and hypotonia, but the infant may progress directly to a deep coma. Hypothermia, tremor, twitching, and seizures can occur. Convulsions may be focal or generalized [19]. Analysis of the urine usually reveals massive ketonuria, and electrolyte analysis indicates a metabolic acidosis. There may be prominent hyperammonemia, notably normoglutaminemic, early in infancy [19–22], but less common than in methylmalonic or propionic acidemias. Hypocalcemia and hyperglycemia may be present [18]. It has been estimated that more than half of the patients die during the first episode very early in life, but the numbers of patients reported are quite small, and the proportion of patients dying undiagnosed early in life may be considerably greater. Intraventricular hemorrhage, cerebral edema, and cerebellar hemorrhage have been described [19, 23, 24]. Vomiting may be so severe that a diagnosis of pyloric stenosis is suspected. Five infants have been treated surgically during the neonatal period, four undergoing pyloromyotomy [2, 17, 25–27]; the fifth was thought to have a duodenal band [16].

The characteristic odor of isovaleric acid may alert the physician to the diagnosis. It has been popularized as the odor of sweaty feet, but it does not smell at all like most locker rooms. It is a pungent, rather unpleasant odor. It can permeate a laboratory working with samples from an acutely ill patient. It was first recognized in the special care nursery when an isolette was opened to examine the baby. It is also important to remember that the odor may be absent at the time it would be most useful, during the acute illness of the first episode. These babies are often born in one hospital, lapse into coma, are treated with parenteral fluids to correct the acidosis or with exchange transfusion for the hyperammonemia, and then transported to a neonatal intensive care unit. By this time, the odor may be undetectable. In some patients, the odor has never been detected, even after the diagnosis was known [28] (Figure 8.4).

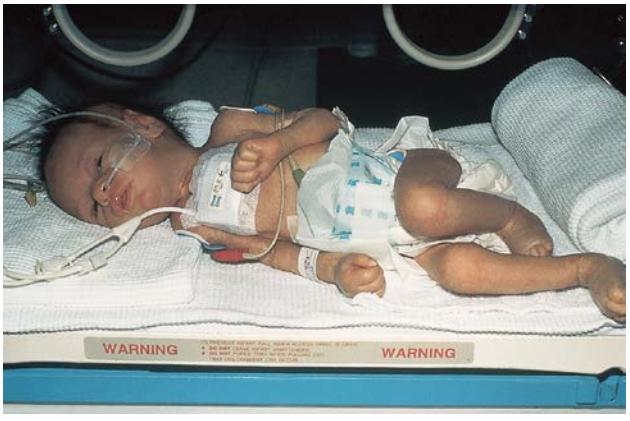

Figure 8.2 AAD: An infant with isovaleric acidemia in the incubator. A nasogastric tube was in place.

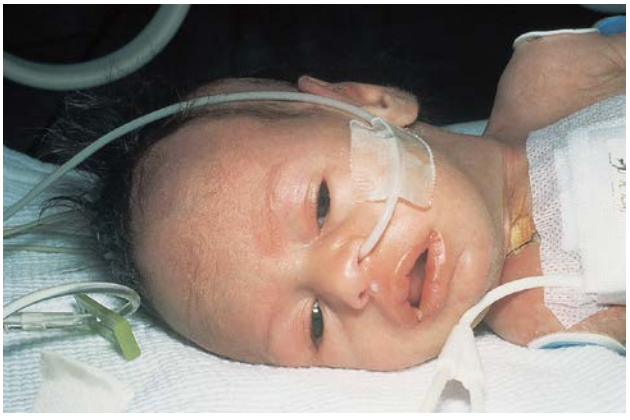

Figure 8.3 AAD: Close up of the face.

Figure 8.4 TM: A seven-year-old girl with isovaleric acidemia. She had microcephaly. The first years of life were characterized by recurrent episodes of acidosis, dehydration, and coma.

Patients who survive the initial episode may have recurrent attacks following infection or surgery in which there is acidosis, ketosis, and coma, much like the initial episode. Vomiting or ataxia may be an initial symptom. The odor may return. Hyperglycemia may occasionally be found as it may in any overwhelmingly ill infant, most likely due to stress-induced hormonal effects, and this in the presence of massive ketosis can lead to a mistaken diagnosis of diabetes mellitus [29, 30]. Treatment with insulin is potentially dangerous in such a patient.

In the chronic intermittent form, patients have a more indolent course presenting first, later in the first year, or later [28, 31, 32]. Episodic disease is associated with intercurrent infection or unusual intake of protein. Episodes decrease in frequency with age, probably as a consequence of decreased frequency of infection. The acute neonatal disease and the chronic, intermittent form of the disease may occur in the same family. A first acute metabolic decompensation of isovaleric acidemia in an adult has been described, and the authors emphasized that internists and adult neurologists need to be aware of organic acidemias [33]. Pancreatitis, both acute and chronic, has been reported as a complication of isovaleric acidemia [34, 35] In three patients, the initial presentation was with pancreatitis. In pediatric patients with pancreatitis, investigation for organic acidemia is prudent. Recently, optic nerve atrophy was also recognized as a long-term complication [36].

Hematologic abnormalities may be prominent, especially in infancy. Leukopenia and thrombocytopenia are common, and in some infants, anemia and a picture of pancytopenia [16, 18–23, 37–39]. These abnormalities may be encountered during the initial or later attacks. The majority of patients surviving the initial episode are developmentally normal (Figure 8.5), but some had mild mental impairment and microcephaly [2, 18, 25, 38, 40, 41], and some severely so [39], and (Nyhan and Barshop, unpublished experience). Hypotonia is common early. Later, there may be ataxia, tremor, dysmetria, extrapyramidal movements, and brisk deep tendon reflexes [39, 40]. Lesions in the basal ganglia lead to dystonia (Figures 8.5 and 8.6). One of the original patients had an unsteady gait as a teenager and had mild mental impairment, but had an uneventful pregnancy and a normal infant [42]. Magnetic resonance imaging (MRI) may be normal, or may show extensive atrophy in an infant with near fatal neonatal illness and cardiac arrest [43]. Neuropathology of the infant dying in the acute episode may show cerebellar edema with herniation [19]. Spongiform changes may be seen in the white matter [16, 44–46], but less prominently than in other organic acidemias or nonketotic hyperglycinemia [46]. Histology of the liver may be that of fatty change [45]. Successful pregnancies and normal infants have been reported in women with isovaleric acidemia [42, 47].

If treatment can be initiated before a first severe metabolic decompensation, the patient's prognosis is significantly improved. Individuals who receive treatment before becoming symptomatic mostly have normal long-term psychomotor development [11, 48] Therefore, early diagnosis is crucial. This is supported by a recent study by Grunert and colleagues, who published details on clinical and neurocognitive outcome in 21 symptomatic isovaleric acidemic patients diagnosed between 1976 and 1999 and results of a systematic review of 155 published cases [49]. They found mild motor deficits in 44 percent and cognitive deficits in 19 percent of patients. The data revealed that the patients' intelligence quotient were not related to the number of metabolic decompensations, but inversely related to the age at diagnosis. In contrast to the high mortality (33 percent) in the literature, only one patient of the study cohort died as a result of metabolic decompensation in the neonatal period. The authors strongly advocate newborn screening for isovaleric acidemia.

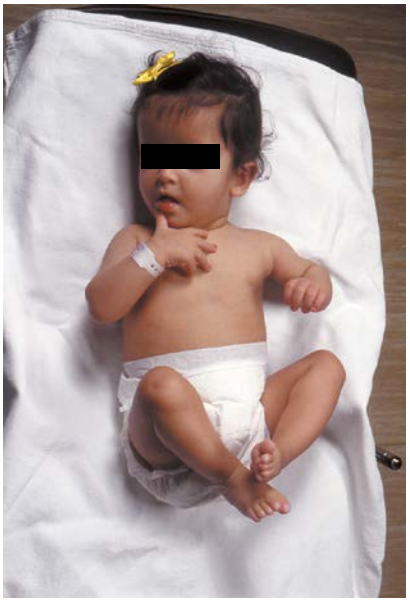

Figure 8.5 AH: A girl with isovaleric acidemia whose course has been entirely benign following the initial episode.

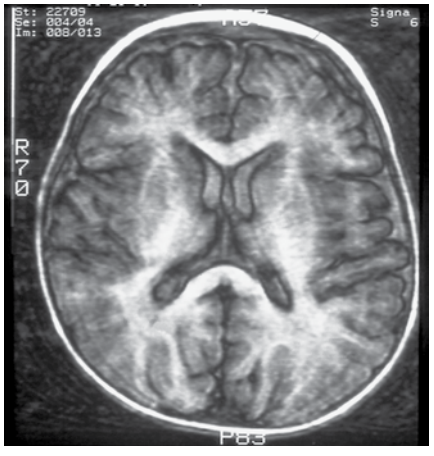

Figure 8.6 Abnormalities in the basal ganglia and white matter disease on T_2-weighted magnetic resonance images.

Special consideration has to be given to the substantial number of patients identified by newborn screening who carry the common mutation (A282V, 932C>T) and are usually completely asymptomatic [10, 11]. Time will tell if this continues to hold true with further experience; the variant enzyme is thermally unstable, which could create a risk during febrile illness [50].

GENETICS AND PATHOGENESIS

Isovaleric acidemia is an autosomal recessive disease caused by deficiency of the mitochondrial enzyme isovaleryl-CoA dehydrogenase. Assay of the enzyme in fibroblasts of a series of patients revealed considerable heterogeneity and residual activity, as much as 13 percent of the control level [51, 52–54]. The enzyme may also be assayed in leukocytes. Prenatal diagnosis has been approached in the same manner [55], however the assay is not generally available. An accurate method for the gas chromatographic-mass spectrometric (GCMS) analysis of 3-hydroxyisovaleric acid [56] or isovalerylglycine permits rapid prenatal diagnosis via direct detection in the amniotic fluid [57]. Isovalerylglycine appears to be the metabolite of choice; it has been diagnostic as early as 12 weeks of gestation. Prenatal diagnosis has also been made by the incorporation of labeled isovaleric acid in chorionic villus material [58], and by electrospray ionization tandem mass spectrometry analysis of acylcarnitines in amniotic fluid (elevated levels of isovalerylcarnitine from 3.12 to 12 μM compared to control values from 0.59 to 0.99 μM) [59], but now predominantly by mutation analysis in informative families.

Isovaleryl CoA dehydrogenase, a member of the acylCoA dehydrogenase family, is made as a 45 kDa subunit precursor [60] and processed to a 43 kDa during import into the mitochondria and then assembled as a tetramer. It is a flavine adenine dinucleotide (FAD)-containing enzyme, whose electrons are transferred to electron transfer flavoprotein (ETF) and transmitted to coenzyme Q of the electron transport chain by ETF dehydrogenase [61].

The isovaleryl CoA dehydrogenase gene (*IVD*) has been located on chromosome 15q14-15 spanning 12 exons over 15 kb [62]. Complementation studies of 12 patients revealed a single group, comprising acute neonatal and chronic, intermittent patients [63]. A certain number of different types of mutation have been described [8] in patients identified because of symptomatic disease. Many have been point mutations in the coding region, leading to an inactive or unstable protein [8, 42, 51, 64]. A few which code for a protein with appreciable enzyme function lead to mild clinical phenotypes [50, 65]. Of two missense mutations, one led to a leucine to proline change at position 13. These mutations lead to mature and precursor proteins of normal size. A single base deletion at position 1179 leads to a frameshift and the addition of eight amino acids, and then a termination leading to a smaller precursor protein. A number of mutations has led to abnormal splicing of the RNA [8], including the deletion of exon 2 and the synthesis of a protein 20 amino acids smaller, which was processed normally into mitochondria [6, 8]. The common missense mutation C932T discovered through newborn screening [10] has been present in homozygous and heterozygous fashion with another mutation. Restriction enzyme analysis with *Bsa*JI yields a recognizable 213-bp uncleaved product, while the wild type is cleaved to a characteristic 46-bp fragment. A 15-bp insertion in intron 7 resulted from missplicing and the use of a cryptic splice acceptor site and maintenance of the correct reading frame [64].

The immediate consequence of the enzyme defect is the elevated concentration of isovaleric acid in the blood. 3-Hydroxyisovaleric acid is also prominent [66]. These elevations are especially true in the acute episode. However, methods for the detection of volatile short-chain acids like isovaleric acid are considerably less than perfect, and mistakes have been made in which the diagnosis of isovaleric acidemia was missed [67]. The way in which the diagnosis can be securely made is by the identification of large amounts of isovalerylglycine in the urine [67–69]. This compound is very stable and is present in the urine even at times of remission and excellent general health. Amounts in the urine may be as great as 3 g/day (2000–9000 mmol/mol creatinine), whereas in normal individuals less than 2 mg is found. A simple screening test has been developed [70], but today most patients are detected by organic acid analysis.

Analysis of organic acids at the time of acute attack reveals the presence of 4-hydroxyisovaleric acid, mesaconic acid, and methylsuccinic acid [71], as well as isovalerylglycine and 3-hydroxyisovaleric acid. Lactic acid, acetoacetic acid, and 3-hydroxybutyric acid are also found in large amounts in the urine. Isovalerylglucuronide has also been identified in the urine [72], and probably represents an additional detoxification pathway. Similarly, isovalerylcarnitine (Figure 8.7) has been identified in the urine [73], and this provides another approach to the diagnosis. After the acute attack has resolved, organic acid analysis usually reveals only elevated isovalerylglycine. Isovalerylglycine can also be identified by nuclear magnetic resonance (NMR) spectroscopy [74].

With the advent of tandem mass spectrometry, we can expect the diagnosis to be made increasingly by the analysis of acylcarnitine profiles either in blood spots on filter paper in programs of newborn screening or in plasma of ill patients. An isolated elevation of C5 acylcarnitine is likely isovalerylcarnitine. 2-Methylbutyrylcarnitine is also C5; its elevation in multiple acyl-CoA dehydrogenase deficiency is accompanied by C4, but isolated 2-methylbutyryl CA dehydrogenase deficiency must be distinguished by the presence of 2-methylbutyrylglycine in the urine or by enzyme assay. In isovaleric acidemia, the ratio of C5 to C3 is useful [75]. False-positive results in newborn screening can result from pivalic acid containing antibiotics or pivalic

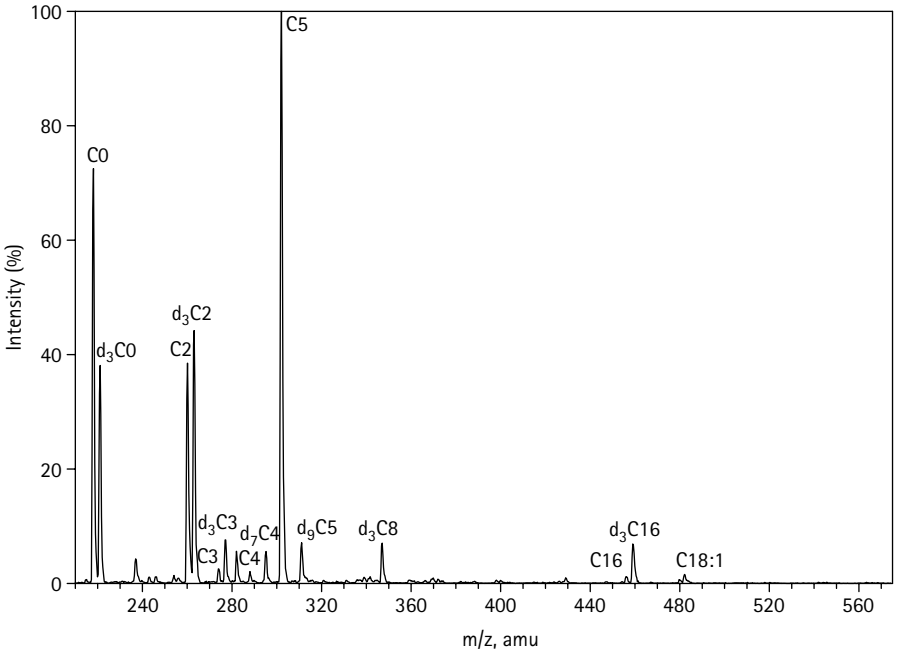

Figure 8.7 Acylcarnitine profile of the blood plasma of a patient with isovaleric acidemia. C5 is isovalerylcarnitine. (Illustration provided by Jon Gangoiti of University of California, San Diego.)

acid derivatives, which are used in the cosmetic industry under the term "neopentanoate" [76].

TREATMENT

The acute episode should be treated vigorously with parenteral solutions of fluid and electrolytes containing sodium bicarbonate and glucose. Provision of energy via oral, nasogastric, or intravenous (IV) routes should be 20 percent to 100 percent above the recommended daily requirements using carbohydrate (such as glucose orally or dextrose 20 percent orally or IV glucose) and fat (intralipid 20 percent). Soluble insulin is provided to avoid hyperglycemia and to support intracellular glucose uptake. The intake of natural protein is stopped for 24 to 48 hours and is then reintroduced gradually as tolerated. The initial episode, especially if complicated by hyperammonemia, may require exchange transfusions or dialysis or the use of benzoate or phenylacetate to promote waste nitrogen elimination. IV carnitine may alleviate hyperammonemia and carnitine deficiency, and it may promote the excretion of isovalerylcarnitine. The administration of exogenous glycine may be helpful in the acute episode [48]. Doses employed have approximated 250 mg/kg per day but can be augmented up to 600 mg/kg per day [77].

Glycine is also employed in chronic therapy with ~150 mg/kg per day [78, 79]. It has been reported to prevent the increase in accumulation of isovaleric acid that follows an oral load of leucine [77]. A dose of 800 mg/day has been recorded for an infant [48]. In an approach to optimal use of glycine supplementation, quantification of isovalerylglycine excretion was studied [80] in two patients with disease of different severity. Different doses were employed, and one was challenged with leucine. Interestingly, the patient with the milder disease excreted much more isovalerylglycine, suggesting that disease severity may be a function of the efficiency of glycine conjugation.

Carnitine may become depleted in isovaleric acidemia. Patients tend to have low levels of free carnitine in plasma and increased losses of esterified carnitine in urine [81–87]. Supplementation restores plasma-free carnitine to normal and increases urinary excretion of isovalerylcarnitine. Studies with isotopically labeled carnitine showed that administered carnitine rapidly enters mitochondrial pools and esterifies with available acyl compounds [73]. Comparison of oral and IV use indicated an oral bioavailability of only 15 percent; IV use is required in acute episodic illness. Oral dosage of 40–100 mg/kg appears adequate for chronic use. There have been conflicting results of studies to determine whether glycine or carnitine is more effective in removing isovaleryl-CoA [82, 85, 86]. It appears prudent to employ both in long-term management.

The cornerstone of long-term therapy is the restriction of the dietary intake of leucine [38]. Our approach to the treatment of organic acidemia is to provide whole protein containing the offending amino acid required for growth and little more. The reduction of leucine intake must be carefully monitored to prevent over-restriction. Our experience with isovaleric acidemia is that the provision of protein can be somewhat more liberal with excellent results (see Figure 8.5) than in other organic acidemias such as propionic acidemia (Chapter 2) or methylmalonic acidemia (Chapter 3). Protein tolerance seems to increase with age, and adult patients usually only follow a moderate protein restricted or vegetarian diet, some even unrestricted [87].

In studies of stable isotopically labeled leucine, more than 90 percent of the excreted metabolites of leucine were

produced by endogenous metabolism when the whole leucine-containing protein intake was 0.75 g/kg [84]. Nutritional therapy should be monitored by quantification of amino acids in plasma ensuring against any one or more amino acids reaching concentrations that would be limiting for growth. Mixtures of amino acids lacking leucine may be employed to increase amino acid nitrogen or nonleucine essential amino acids. Supplementation with alanine may accomplish a similar goal [88].

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9

Glutaric aciduria (type I)

MAJOR PHENOTYPIC EXPRESSION

Megalencephaly; acute encephalitis-like crises; neurodegenerative disorder with spasticity, dystonia, choreoathetosis, and dyskinesia; seizures; characteristic temporal hypoplasia, acute subdural hemorrhages or subdural fluid collections and striatal degeneration; glutaric aciduria and 3-hydroxyglutaric aciduria; and deficient activity of glutaryl CoA dehydrogenase.

INTRODUCTION

Glutaric aciduria was first described by Goodman *et al.* [1] in two siblings who began at three and seven months of age to have a neurodegenerative disorder characterized by opisthotonos, dystonia, and spasticity. One had a chronic compensated metabolic acidosis in which the serum bicarbonate concentration ranged from 7.5 to 15.7 mEq/L. It has now become apparent that macrocephaly is a prominent, often the initial, manifestation in infancy [2, 3].

The cause of this disease is deficiency in the activity of glutaryl CoA dehydrogenase. This enzyme is on the pathway for the catabolism of lysine, hydroxylysine, and tryptophan (Figure 9.1). This pathway is also the site of the defect in 2-oxoadipic aciduria [4].

The disorder provides an argument for organic acid analysis in patients with dystonic cerebral palsy [3] and with megalencephaly. Diagnostic difficulty in infancy is highlighted by the fact that glutaric aciduria may be absent, even at times of acute neurologic decompensation [3, 5]. Some patients are identified by the presence of 3-hydroxyglutaric acid rather than glutaric acid in the urine [6, 7]. Analysis of organic acids in the cerebrospinal fluid (CSF) [5, 8], mutation analysis or enzyme assay may be required for diagnosis. The presence of glutarylcarnitine in blood or urine may also be diagnostic, and the assay of blood spots forms the basis for newborn screening [9, 10].

Glutaryl CoA dehydrogenase has been mapped to chromosome 19p13.2 [11]. The gene contains 11 exons over 7 kb [12]. More than 200 mutations have been identified and most patients are heterozygous for two different mutations [13]. Mutations, common in inbred populations, are IVS-1 +5G>T mutation in Indians in Island Lake, Canada [14] and p.Ala421Val in the Old Order Amish in Lancaster County, Pennsylvania [12].

CLINICAL ABNORMALITIES

Progressive megalencephaly may be present at birth [3] and may necessitate cesarean section, or it may develop in the first weeks or months of life [2, 3]. Initially, affected babies show relatively mild physical signs such as muscular hypotonia with prominent head lag, high palate, feeding difficulties and irritability [15–17]. All these symptoms are reversible and of little prognostic significance. By six months, head circumference may be well above the 98th percentile [2] or 2–5 SD above the mean [3]. At this time, magnetic resonance imaging (MRI) or computed tomography (CT) may reveal the characteristic findings of temporal hypoplasia (95 percent of all patients; [Figure 9.2]), wide anterior temporal and sylvian CSF spaces, subependymal pseudocysts, an immature gyration pattern, delayed myelination, and isolated T_2 hyperintensity in the globus pallidus. Temporal hypoplasia may develop *in utero* in the third trimester. The neuroradiologic studies are usually ordered to rule out hydrocephalus, and they do. Macrocephaly is not found in every patient, but in a series

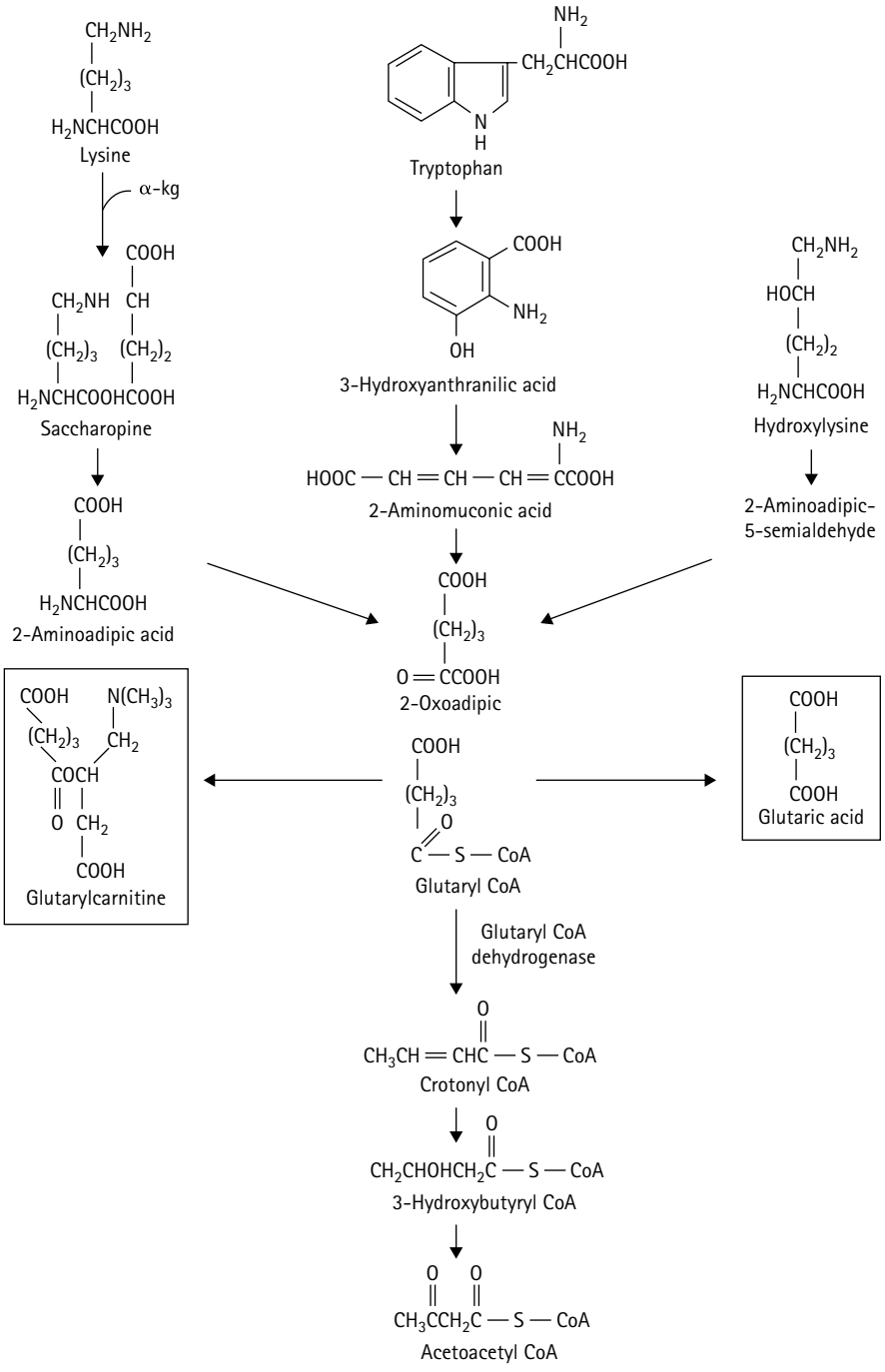

Figure 9.1 Catabolic pathways for lysine and tryptophan and the site of the defect in glutaryl CoA dehydrogenase.

of 11 infants [3] it was present in all but two, and these two never had an acute encephalopathic crisis. A real clue to early diagnosis is the crossing of percentiles for head growth; this acceleration is maximal at three to nine months.

Patients with or without macrocephaly develop almost normally until the majority of untreated patients develop an acute neurologic presentation, which may be at two to 37 months. The mean age of onset of the encephalopathic episode is nine months. However, it is now clear that many of these infants considered to be presymptomatic may have axial hypotonia, jitteriness, irritability, or vomiting. The acute encephalopathic episode is often preceded by an infection and often accompanied by fever, so that an initial diagnosis of encephalitis is commonly made. Other possible triggers are fasting, routine immunizations or minor trauma. The episode is characterized by acute loss of functions, such as head control, sucking and swallowing reflexes, and the ability to sit, pull to standing, or grasp toys [15–18]. Often, the infant is still alert with profound hypotonia of the neck and trunk, stiff arms and legs, and dystonic or twisting (athetoid) movements of hands and feet, especially when agitated. There may be convulsions and paroxysmal abnormalities of the EEG. An increase in CSF concentration of protein may further suggest a diagnosis of encephalitis [19]. Recovery from the acute episode is slow and incomplete, leaving evidence of developmental

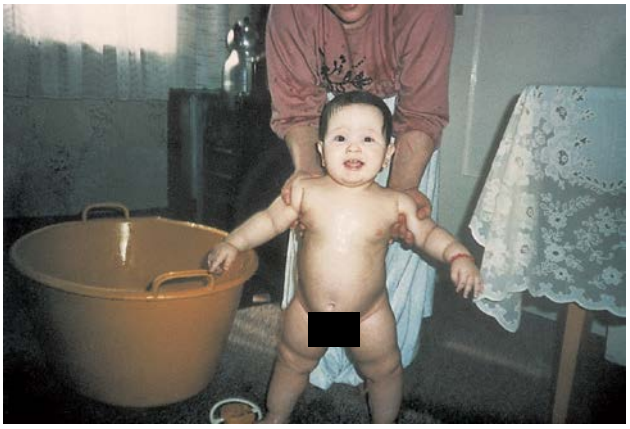

Figure 9.2 SS: An eight-month-old infant with glutaric aciduria. She was macrocephalic from birth, but seemed otherwise well prior to encephalopathic crisis. A black and white version was published in *Pediatrics* [3].

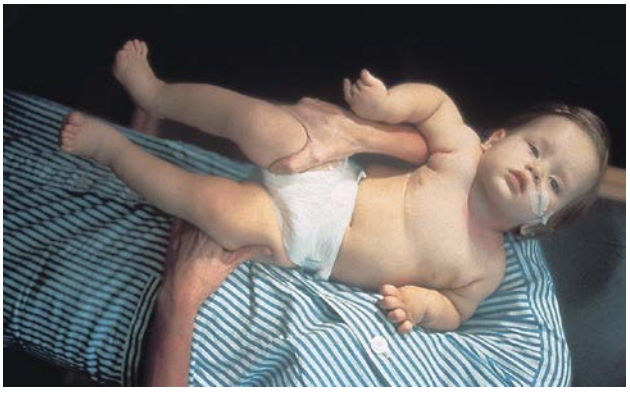

Figure 9.3 SS: At 14 months, after encephalopathic crisis, leaving the child severely handicapped. She succumbed to aspiration pneumonia at 33 months. A black and white version was published in *Pediatrics* [3].

deficiency and dystonia or dyskinesia (Figures 9.2–9.11) [15–24]. There may be hypotonia, grimacing, opisthotonos, rigidity, clenched fists, or tongue-thrusting. There may be repeated episodes associated with catabolic situations, in which each is followed by further evidence of neurologic deterioration. Cognitive function may initially be spared, but progressive impairment may occur. Some patients do not have acute episodes; instead, the course is one of slow neurologic degeneration. In analogy to patients with acute encephalopathic crises, patients with so-called insidious onset type develop striatal damage without preceding acute events. Originally, the frequency of insidious onset was estimated to be 15 percent of patients, but was up to 50 percent in more recent publications. The ultimate picture of spastic, dystonic cerebral palsy and mental deficiency is the same. With aging, movement disorders tend to evolve from mobile to fixed dystonia associated with akinetic-rigid Parkinsonism [25]. Though chronic epilepsy is rare, many children will have sudden dystonic spasms that could be mistaken for seizures [26]. On the other hand, the course

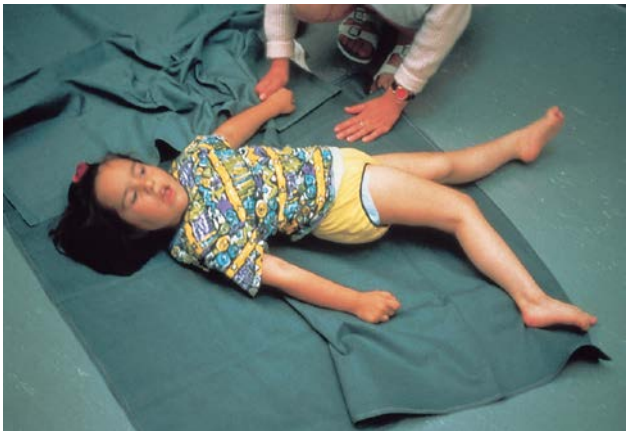

Figure 9.4 JS: An unrelated patient with glutaric aciduria who had been macrocephalic at birth, head circumference 39.5 cm developed normally until 3 years of age [30]. During the course of a febrile bronchitis, she suddenly lost the ability to walk, stand or talk. Because of choreic movements and character changes, a diagnosis of Sydenham chorea was made. During the following two months she regained some skills, such as being able to stand again and speak a few words, when she deteriorated in the course of a febrile gastroenteritis. Thereafter, she was neurologically devastated.

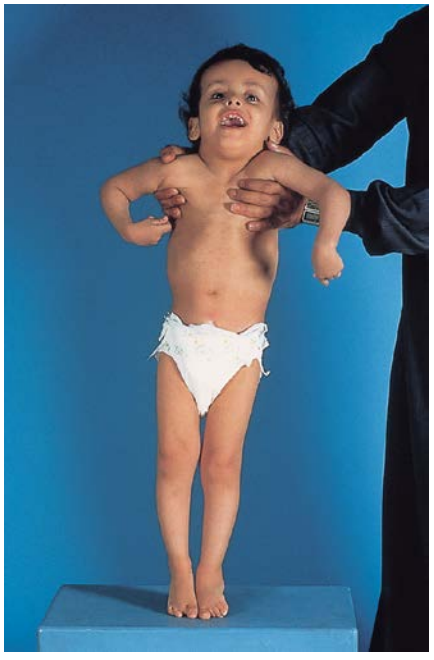

Figure 9.5 AS: An 18-month-old boy with glutaric aciduria illustrating the dystonic posturing and facial grimacing. He had developed normally until an initial episode at seven months during which the CSF protein was 500 mg/dL.

may be quite variable, even among siblings; for instance, one sibling at four years could not sit, while his eight-year-old brother was doing well in school [20]. At the other extreme, two asymptomatic adult homozygous individuals have been observed [21, 27], but these patients also had neuroradiographic evidence of frontotemporal atrophy.

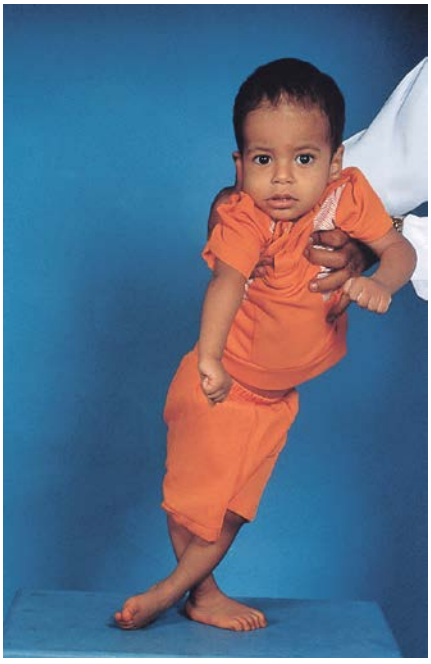

Figure 9.6 AT: A 15-month-old boy with glutaric aciduria. He was dystonic and the legs scissored.

Figure 9.7 AM: This 20-month-old girl had spastic quadriplegia and oposthotonic posturing of the head.

We have studied [6] siblings with pronounced dystonia who were intellectually normal and had normal MRI scans of the brain.

In recent years, adult onset has been described in adolescents or adults presenting with peripheral neuropathy or chronic neurologic deterioration and psychiatric illness and leukoencephalopathic changes of different degree [28, 29], raising the possibility of a distinct clinical manifestation of glutaric aciduria in adolescence/adulthood (Figure 9.17).

Profuse sweating is another common manifestation in neurologically impaired patients [3]. Some patients have had repeated episodes of unexplained fever (hyperpyrexic crises), irritability, ill temper, anorexia, and insomnia. Some have had hepatomegaly, and fasting hypoglycemia

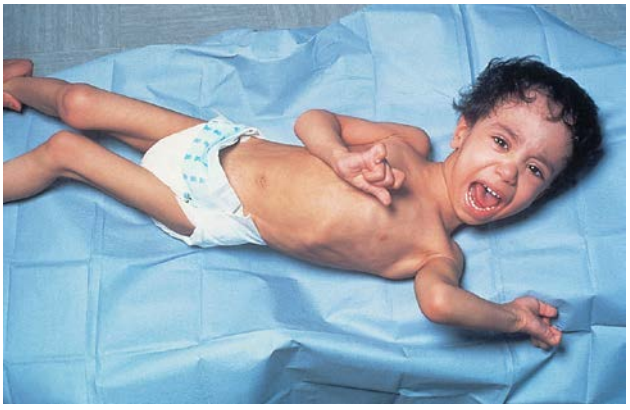

Figure 9.8 FQOM: A 15-month-old boy with glutaric aciduria. Dystonic posturing was associated with athetoid movements of the hands.

Figure 9.9 A 47-year-old Saudi man with glutaric aciduria. He came to attention because of a cousin with the disease and severe dystonia, chorea, and opisthotonus. This man, her uncle, had some dystonia of the hands on intention or excitement and imperfect gait. He had glutaric aciduria and the classic tandem mass spectrometry findings. The mutation found by Dr S Goodman of the University of Colorado: a leucine 179 arginine which has not been reported in another family. Two children in this family were diagnosed as having the disease, but have had no neurologic abnormalities.

can occur in older children and adults. Death in a Reye-like syndrome has been reported [22]. Death often occurs before the end of the first decade [17, 20, 23, 24, 31].

Episodes of metabolic imbalance, ketoacidosis, or hypoglycemia that characterize most organic acidurias do not generally occur in this disorder. Low levels of bicarbonate may be seen chronically [1] or during acute episodes of illness [3], but in most patients, they have always

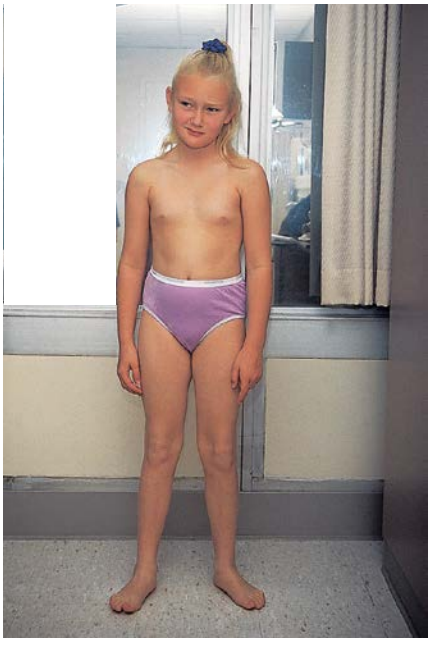

Figure 9.10 A nine-year-old girl with glutaryl CoA dehydrogenase who excreted 3-hydroxyglutaric acid [6]. Photographed walking, she illustrated a wide-based, dystonic gait.

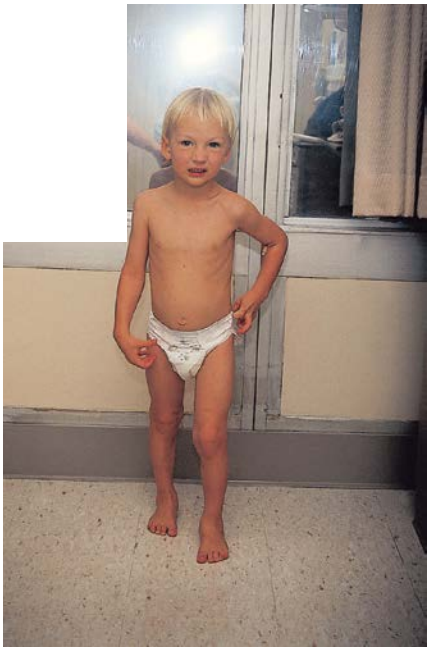

Figure 9.11 The three-year-old brother of the girl shown in Figure 9.10 displayed dystonic grimacing, athetoid posturing of the arms and hands, and a somewhat broad gait.

been normal [16, 23, 24]. Rarely, there may be ketosis, hypoglycemia, hyperammonemia and elevated levels of transaminases in the blood during the acute episodes [16, 19, 21–24, 27].

An unusual clinical occurrence is rhabdomyolysis, as seen in disorders of fatty acid oxidation [32]. The patient

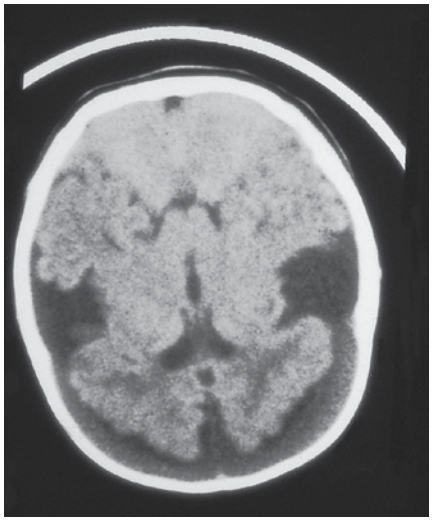

Figure 9.12 CT scan of the brain of a 13-month-old infant with glutaric aciduria. The patient was presymptomatic, but there was extensive frontotemporal atrophy.

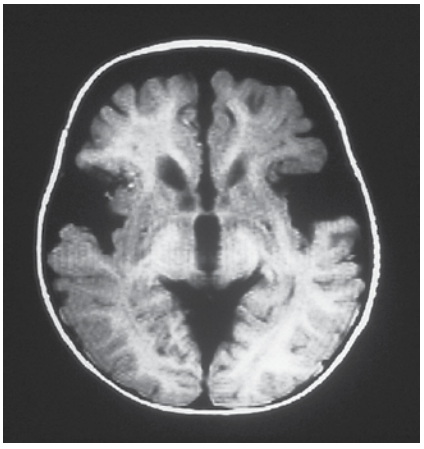

Figure 9.13 Magnetic resonance image of the patient in Figure 9.5, illustrating extreme loss of volume, striatal atrophy and the pattern of frontotemporal atrophy.

reported had three episodes, the last one fatal. Levels of creatine kinase ranged from 78,000 to 189,000 IU.

Neuroradiographic findings document the region- and age-specific pathology of glutaric aciduria [33]. Temporal hypoplasia on CT or MRI with increased CSF-containing spaces in the sylvian fissures and anterior to the temporal lobes (Figures 9.12–9.14, 9.16 and 9.17) [3, 21, 34, 35] may be manifest *in utero* or may occur in infancy antedating neurologic symptoms [3, 34]. In addition, an immature gyration pattern, delayed myelination, subependymal pseudocysts, and isolated T_2 hyperintensity in the globus pallidus may be seen prior to the changes in the basal ganglia (Figure 9.14) [34]. The clinical significance and long-term prognosis of these MRI findings are still uncertain and reversible after early diagnosis and reliable treatment (Figure 9.15). Subdural collections of fluid have been observed in a number of patients (Figure 9.16) [34, 36, 37].

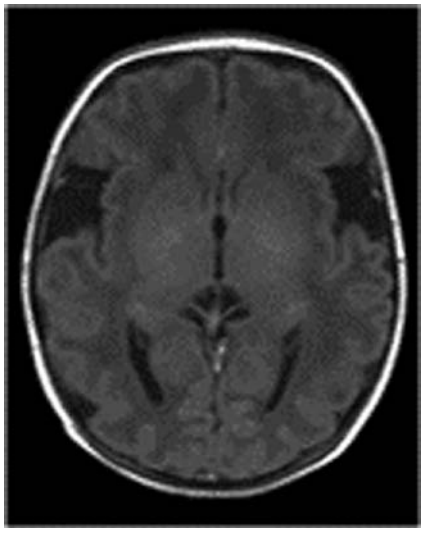

Figure 9.14 **T_1-weighted** axial MRI of an asymptomatic newborn with glutaric aciduria type I identified by newborn screening showing enlargement of temporo- and frontopolar CSF spaces and immature gyration pattern.

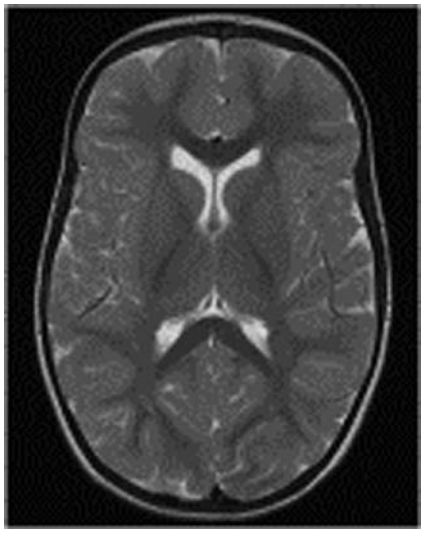

Figure 9.15 T_2-weighted axial MRI of the same child at age 2 years showing complete resolution of the initial MRI abnormalities under continuous metabolic therapy.

There may be hygromas or actual subdural hematomas [36, 37], because of rupture of bridging veins stretched by the enlargement of these spaces. These occurrences have given rise to a suspicion of child abuse. Retinal hemorrhages may add to this suspicion. Certainly, this disease is not the most common cause of this syndrome resembling nonaccidental trauma, but it is reasonable to be sure to exclude glutaric aciduria in any such patient without other obvious signs of trauma. After implementation of newborn screening programs, subdural hygromas have been reported less often in the literature.

Ventriculomegaly and basal ganglia lesions develop after the encephalopathic crisis and are indicative of

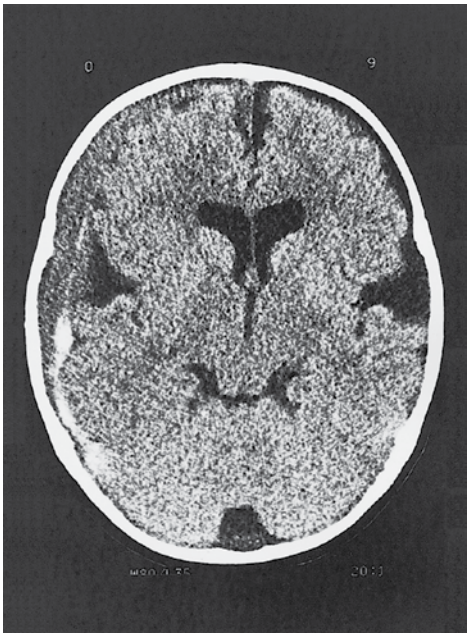

Figure 9.16 CT scan of a patient at nine months, illustrating the development of a chronic subdural hematoma. Under continuous metabolic therapy these lesions resolved without permanent damage.

permanent damage [33–35] (Figure 9.13). Damage and finally atrophy of the putamen and caudate, as well as ventriculomegaly are strongly correlated with permanent neurologic deficits, especially a movement disorder. White matter changes are variable but frequently present, tend to increase from younger to older age and may become recognized as important long-term complications [28, 33] (Figure 9.17). The increased signal intensity seen on T_2- and FLAIR-weighted images is localized mainly within the periventricular white matter but also in the subcortical U-fibers. Since white matter abnormalities are the predominant neuroradiologic finding in non-dystonic adult patients, it remains to be elucidated whether adult-onset type glutaric aciduria type I should be regarded as a distinct disease variant.

The neuropathology [35, 38, 39] is that of extensive striatal neurotoxicity. There is neuronal loss and astrocytic proliferation in the caudate nucleus and the putamen and, in some, the globus pallidus. Changes tend to be more extensive in older patients [39]. Prominent spongiform change is seen predominantly in the white matter. Despite the cortical atrophy reported on imaging studies, neuronal loss was not found in the cortex. There may be microvesicular lipid in the liver.

The natural history and outcome in this disease has been assessed by an international group [17] that assembled data on 279 patients, 185 of whom were diagnosed after a clinical presentation and 61 of whom were diagnosed presymptomatically – 23 by newborn screening, 24 by high-risk screening, and 14 because of macrocephaly. Some highlights included the fact that the first crisis usually

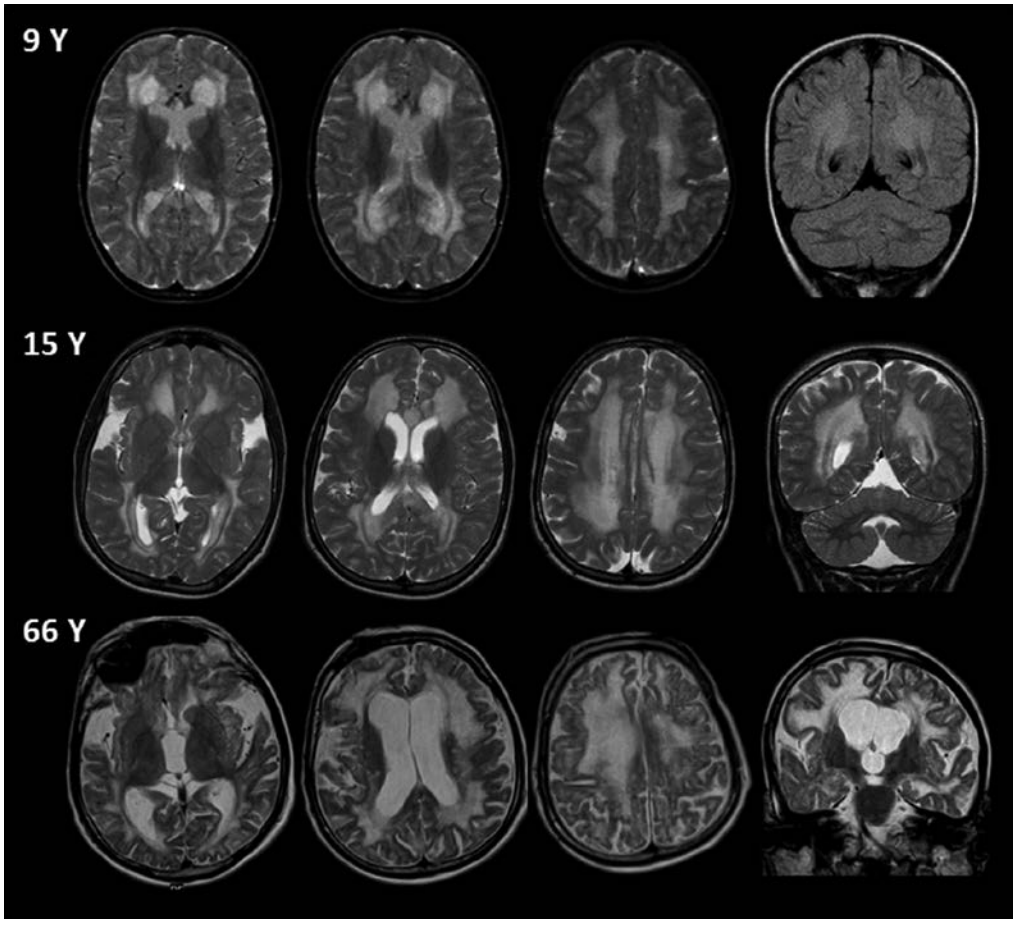

Figure 9.17 Cranial MRI studies in patients with presumed late-onset type glutaric aciduria type I at different ages. Patients were asymptomatic during infancy and early childhood. T_2-weighted MRI scans showed confluent supratentorial hyperintensities of white matter spreading from the periventricular zone to the U-fibers. Basal ganglia remained unaffected. Dilation of extracerebral CSF spaces and ventricles were variable. "Leukodystrophy" was suspected (With courtesy of Drs Inga Harting and Angelika Seitz, Department of Neuroradiology, University Hospital Heidelberg, Germany).

occurred in infancy; actually, 95 percent by the age of two years; the median age was nine months. The oldest age at which a repeat crisis occurred was 70 months, indicating the importance of focusing on their prevention during the first six years of life. These data were similar to the experience of Strauss and colleagues [15] with 77 personally observed patients over 14 years, 78 percent of whom were diagnosed after the development of striatal necrosis, the onset of which was between two and 18 months; no child in this series developed basal ganglia injury after the second birthday.

Among 49 children in the International Cross-sectional Study who died, the median age was 79 months (range, 5–490 months). The 50 percent estimated survival time was 25 years; death was most commonly as a result of aspiration pneumonia. In general, the younger the age of onset at first crisis, the more likely was a patient to die. Also, neuroimaging evidence (basal ganglia degeneration and enlargement of CSF-containing spaces) were indicative of a poor outcome [33]. Chronic renal failure was recently recognized in a number of adolescent and adult patients [40], the mechanism of which remains to be determined.

GENETICS AND PATHOGENESIS

The site of the molecular defect is in glutaryl CoA dehydrogenase (see Figure 9.1). Activity is most commonly measured in fibroblast or leukocyte lysates in which residual activity is virtually undetectable [21, 41]. The disease is transmitted as an autosomal recessive trait. Intermediate activities of the enzyme have been documented in leukocytes and fibroblasts of heterozygotes [21], and consanguinity has been observed [19–21].

The enzyme (EC 1.3.99.7) is a mitochondrial homotetrameric, flavin adenine dinucleotide (FAD)–binding dehydrogenase. Its electrons are transferred to ubiquinone in interactions with the electron transfer flavoprotein (ETF) and its dehydrogenase (ETF: ubiquinone oxidoreductase). FAD is bound to the enzyme. Paper chemistry would indicate that glutaconylCoA was an intermediate in the reaction, but if so, it must remain bound to the enzyme because the only products of labeled substrate are crotonylCoA and CO_2 [42, 43]. The enzyme defect results in an accumulation of upstream metabolites,

especially glutaryl-CoA, which is subsequently converted to glutaric acid and 3-hydroxyglutaric acid.

The cDNA for the enzyme has been cloned and sequenced. There are 11 exons. The gene has been mapped to chromosome 19p13.2 [11]. The mutation (IVS-1+5G>T) that has been found in homozygous Indians in Manitoba, in a population in which glutaric aciduria is common, is a splicing mutation [14, 44, 45]. The G to T transversion in intron 1 at position +5 changes a donor splice site to Ggtcatt, which permits variable splicing, some normal but most using a cryptic donor site, 26 bases upstream and leading to a deletion of 26 base pairs, removing eight amino acids and causing a translational frameshift. Variable amounts of normal and truncated mRNA in varying individuals would determine variable phenotypes. In this population, excretion of the key metabolites is so low that patients cannot be reliably diagnosed by gas chromatography mass spectrometry (GCMS) or tandem mass spectrometry (MS/MS). In the Amish population, in which glutaric aciduria is also common, a C to T change at 1298 changes the alanine at 421 to valine [12].

The most common mutation in the broader population was c.1240C>T, which changed arginine at 402 to tryptophan (p.Arg402Trp). This mutation was found in 30 percent of alleles in Spain and in 40 percent of those in Germany [46, 47]. Expression of various mutations in *E. coli* led to enzyme activities ranging from less than 17 percent of normal activity to 20 percent in the Amish mutation.

Correlations between genotype and clinical severity have been elusive, possibly because neurologic impairment is related to the occurrence of encephalopathic crises rather than any other clinical, biochemical, or molecular feature. However, certain mutations have correlated well with high excretion of glutaric and 3-hydroxyglutaric acids, and others have been found in patients with low excretion [46]. In the former group, the most frequent mutations were p.Arg402Trp and p.Ala293Trp resulting from a c913G>A change in exon 8. These mutations were found in the low excretor group only in heterozygosity, especially in combination with p.Arg227Pro or p.Val400Met which together accounted for over half of the mutant alleles in the low excretor group [46–48]. Among eight families identified in Israel, six were of Moslem origin, and two non-Ashkenazi Jews, and eight previously unidentified mutations were found including a 1-bp deletion at 1173 [49]. The siblings with 3-hydroxyglutaric aciduria [6] were compounds of p.Arg227Pro and p.Glu365Lys.

Defective enzyme activity leads to glutaric aciduria, the feature by which the diagnosis is usually made. The amounts reported may be massive: 850–1700 mmol/mol creatinine [1] and 900–1200 mmol/mol creatinine [3]. Normal levels of glutaric acid in urine range from 1.1 to 8 mmol/mol creatinine [7]. However, patients with smaller amounts (80–200 mmol/mol creatinine) [3, 7] have been observed, and many patients have been reported in whom glutaric acid and other characteristic metabolites were not found in the urine [7, 14], at least until investigated with stable isotope dilution techniques. Metabolites in the urine have also been observed intermittently [50]. The other characteristic metabolites found in the urine are 3-hydroxyglutaric and glutaconic acids [19]; amounts are usually less than those of glutaric acid. On the other hand, we have seen children with documented deficiency of the enzyme in whom only 3-hydroxyglutaric was found in the urine, in the absence of accumulation of glutaric acid [6, 7]. Excretion of glutaconic acid may exceed that of 3-hydroxyglutaric acid only in an acute ketotic episode when the urine also contains 3-hydroxybutyric, acetoacetic, adipic, suberic, and sebacic acids.

Levels of glutaric acid in plasma have ranged from 5 to 18 μmol/L [7], but normal levels (0.6–3 μmol/L) have also been recorded. Without stable isotope dilution techniques, glutaric acid is undetectable in normal plasma or CSF [7]. In patients, levels of glutaric acid in the CSF have ranged from 20 to 40 μmol/L [3, 5, 7]. The CSF may be the only fluid in which elevated levels are found [5]. Glutaric acid concentrations have been found to be elevated in all tissues examined [51]. Intracerebral concentrations of glutaric and 3-hydroxyglutaric acids exceeded plasma concentrations by 10- to 1000-fold.

Measurement of bound glutaric acid by organic acid analysis following mild alkaline hydrolysis may indicate the diagnosis in patients with normal urinary glutaric acid [7]. This is probably a reflection of the excretion of glutarylcarnitine, which may be detected by MS/MS.

The analysis of glutarylcarnitine in blood spots has been incorporated into many programs of expanded newborn screening [10]. There are cautions about the possibility of false negatives. A reported infant with glutaric aciduria was missed in a neonatal screening program [9]. Actually, there was glutarylcarnitine on the initial spot, but a repeat was normal, and the patient was only identified after developing dystonia at 11 months during an intercurrent infection. That state has since increased the sensitivity of the screen for this disease by adjusting the signal ratio cutoff, and now recommends a complete work up for any positive rather than a repeat screening analysis of a blood spot. With time, amounts of acylcarnitines may decrease as carnitine stores are depleted. In addition, patients with mutations which put them in the low excretor group [14, 45] have been tested for glutarylcarnitine in blood spots and gave negative results even in the presence of carnitine supplementation.

Diagnostic confusion is symbolized by the fact that classic patients may excrete no elevated glutaric acid at all, and even 3-hydroxyglutaric excretion may be normal. On the other side of the coin, elevated excretion of glutaric acid (100–150 mmol/mol creatinine) has been reported [52] in a patient found not to have glutaryl CoA dehydrogenase deficiency; antibiotic treatment abolished the glutaric aciduria; so, the source must have been intestinal bacteria. In addition, elevated glutaric acid may reflect (transient) renal failure. Patients with 2-oxoadipic/2-aminoadipic aciduria have also been given the diagnosis of glutaric aciduria type I because of decarboxylation of 2-oxoadipic to glutarate

during processing for organic acid analysis. However, the level of 3-hydroxyglutarate is normal in these cases. A small number of patients were classified as glutaric aciduria type III, with constantly elevated excretion of glutaric acid but normal excretion of 3-hydroxyglutaric acid, normal levels of glutaryl-carnitine and no apparent neurologic disease [53]. This constellation was also recognized in the Old Order Amish of Lancaster County, Pennsylvania, during population screening for glutaric aciduria type I. Mutations in the *C7ORF10* gene located on 7p14 were identified as causative for glutaric aciduria type III [54]. An additional confounder is ketosis, which has been reported [55] to cause significant increases in the excretion of 3-hydroxyglutaric acid in the urine of patients who did not have glutaryl CoA dehydrogenase deficiency. SCHAD deficiency should be considered as another cause of elevated 3-hydroxyglutaric acid. In two patients with glutaryl CoA dehydrogenase deficiency and no elevation of glutarylcarnitine in the blood, there was a sizeable excretion of glutarylcarnitine in the urine [56]. False-positive newborn screening may be caused by so far undiagnosed maternal glutaric aciduria type I. There is sufficient diagnostic uncertainty that enzyme analysis or the identification of a mutant gene is an essential criterion for diagnosis.

Most patients have low concentrations of free carnitine in plasma and elevated levels of esterified carnitine, especially in urine [3, 15–17, 21]. Low muscle carnitine has been reported [21], even in an asymptomatic patient.

The incidence of the disorder has not been known, until experience with newborn screening in North America, Australia, Germany, and Taiwan gave an incidence of one in 106,900 [10]. Increased frequency of the disease up to 1 in 250 newborns has been observed in Ojibway Indians in Manitoba [44] and in the Amish of Lancaster County, Pennsylvania [15, 57]. In Manitoba Indians, DNA-based screening gave an incidence of one in 300 [56]. Carrier detection has been improved by assay of the enzyme in cultured interleukin-2-dependent leukocytes [58], but there was still some overlap between controls and obligate heterozygotes. Molecular analysis for mutation is the most reliable method of carrier detection. Prenatal diagnosis has been made by the detection of increased amounts of glutaric acid [59] in amniotic fluid, as well as by assay of the enzyme in cultured amniocytes. Molecular analysis for mutation is the most reliable method of prenatal diagnosis. The value of prenatal diagnosis has been questioned [21] on the basis of the existence of asymptomatic homozygotes, but these individuals had frontotemporal atrophy, and studies of intellectual function were not reported.

Considerable attention has been devoted to pathogenesis and the extraordinary vulnerability of the striatum, particularly the caudate and putamen. It has seemed likely that the accumulation of metabolites and something about the catabolic response to acute infection are relevant to neuronal damage. Glutaric acid and its metabolites are produced endogenously in the CNS and accumulate because of limiting transport mechanisms across the blood-brain barrier (trapping hypothesis) [60]. The similarity of structures of glutaric and glutamic acids, and the fact that glutaric and 3-hydroxyglutaric acids inhibit glutamate decarboxylase of brain [61] has led to an excitotoxic theory of neuronal damage in this disease. In striatal slice cultures, 3-hydroxyglutaric acid induced neuronal degeneration by activation of NMDA receptors [62]. Furthermore, glutaric and 3-hydroxyglutaric acids indirectly modulate glutamatergic and GABAergic neurotransmission, resulting in an imbalance of excitatory and inhibitory neurotransmission. Convulsions and striatal neuronal damage were caused in rats by direct striatal injection of 3-hydroxyglutaric acid [63]. Finally, glutaryl-CoA inhibits 2-oxoglutarate dehydrogenase complex in analogy to succinyl CoA [64] and by that the Krebs cycle.

TREATMENT

Treatment with carnitine and the prompt, vigorous intervention in intercurrent illness with the provision of energy from glucose, water, and electrolytes has been shown to prevent striatal degeneration [15–17, 40, 16]. A protocol we have employed (Table 9.1) was derived from the large experience of Morton with the glutaric aciduria of the Amish. Some have added insulin to the regimen and it is likely that we will as well. We use intravenous carnitine in a dose of 300 mg/kg. The initial dose for chronic oral carnitine administration approximates 100 mg/kg, and we adjust dosage dependent on intestinal tolerance and urinary carnitine ester excretion.

Implicit in programs of newborn screening is the expectation that treatment will prevent encephalopathic

Table 9.1 Management of acute imbalance in glutaric acidemia I

Time (hours)		mL/kg
0–1	Intravenous bolus 5 percent dextrose in Ringer lactate + 2 mEq/kg $NaHCO_3$	20
1–24	Intravenous 12.5 percent dextrose 20 mEq/L KCl, 50 mEq/L $NaHCO_3$, 50 mEq/L NaCl; i.v. carnitine 300 mg/kg; insulin may be added in dosage of 0.05–0.1 μg/kg per hour monitoring blood glucose and electrolytes	140
	For vomiting, 0.15 mg/kg i.v. Zofran, may repeat in 4–8 hours. Alternatively, Kytril 10 μg/kg i.v.	
	Calorimetry – provide CHO at least 1.5 × BMR	

Patients have gastrostomy placed on diagnosis. Parents are instructed to stop protein and begin administration of calories and water on the way to the hospital.

neuronal damage. Experience to date suggests that this can be the case [10, 15–17, 40, 64, 65]. The occurrence of frontotemporal atrophy at birth implies restriction of any postnatal therapeutic effects, but there is even evidence that this too may improve and even resolve completely [18, 34]. We reported on 38 patients identified by newborn screening and receiving intensive management [18] that encephalopathic crises were absent in 89 percent of these infants prospectively treated, while in a historical cohort, 90 percent of patients developed encephalopathic crises [15, 17, 66]. Experience from the same group [67] indicates that some genotypes may lead to acute encephalopathy despite adherence to all of the current mainstays of treatment. An infant, homozygous for E365K, experienced such an episode and despite treatment was left with a dystonic, dyskinetic movement disorder, and characteristic striatal lesions on MRI [66].

A diet low in tryptophan and lysine will decrease the excretion of glutaric acid in urine to one-third or more [1, 3, 15–18, 68] of the usual values, but clinical improvement resulting from diet alone has been little or none in patients who have had an encephalopathic crisis. Information from the international study [17] indicated clearly that in presymptomatic patients, treatment is effective. The data indicated carnitine effective in preventing secondary carnitine deficiency and in acting as a detoxifying agent. A lysine-restricted diet was found to be more effective than a protein-restricted diet. Patients receiving a lysine-restricted diet should be supplemented with a lysine-free, tryptophan-reduced AminoAcidMixture fortified with minerals and micronutrients. Additional fortification of the diet with L-arginine, which competes with lysine for transport at the blood-brain barrier, is currently under investigation with promising results [68]. An international working group published and updated guidelines for the diagnosis and management of this disease [69]. The recommendations are for a strict adherence to the emergency protocol, a diet restricted in lysine intake to the "minimum requirements", which in our view are generous, plus supplementation with lysine-free, tryptophan-reduced amino acid mixtures and supplementation with carnitine. The most frequent mistake and, thus, the greatest risk to suffer acute and permanent basal ganglia damage, is a delayed or improper emergency treatment in infancy and early childhood. Parents should be instructed to call their doctor if the child develops fever of 38.5°C, signs of infection, or irritability. During the vulnerable period of brain development, i.e. 0–5 years, encephalopathic crisis can develop in hours to minutes without gradual or even significant metabolic derangement.

Riboflavin, as the coenzyme of the dehydrogenase, has appeared logical, and 100–300 mg/day have been used [69], but without clear evidence of therapeutic effect. Riboflavin was pronounced ineffective by the international consortium [17, 69]. Low concentrations of GABA in the basal ganglia led to the use of the GABA analog 4-amino-3-(4-chlorphenyl)butyric acid (baclofen, Lioresal); results have usually not been impressive, but improvement was reported in two of three patients in a double-blind controlled study given 2 mg/kg per day [9]. Diazepam (0.1–1 mg/kg daily) is commonly used to reduce involuntary movements and improve motor function. Use and dosage are limited by worsening of axial hypotonia. Trihexyphenidyl can be efficacious and can be used safely in adolescence in high dosage for segmental and generalized dystonia. Botulinum toxin and intrathecal baclofen are valid additions for focal dystonia or very severe dystonia, especially if accompanied by spasticity [69]. Valproic acid has been recommended, but most feel this drug is contraindicated [3, 69].

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3-MethylcrotonylCoA carboxylase deficiency/ 3-methylcrotonylglycinuria

MAJOR PHENOTYPIC EXPRESSION

Reye-like episodes of ketoacidosis, hypoglycemia, hyperammonemia, and coma; seizures, failure to thrive, excretion of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid; and deficiency of 3-methylcrotonylCoA carboxylase. An increasing population of asymptomatic individuals, many of them adult women discovered because of elevated 3-hydroxyisovalerylcarnitine detected in the neonatal screening blood spots of their infants.

INTRODUCTION

3-MethylcrotonylCoA carboxylase (EC 6.4.1.4) deficiency (Figure 10.1) is a disorder of leucine catabolism in which elevated quantities of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine are found in the urine. The disorder is often referred to as isolated 3-methylcrotonylCoA carboxylase deficiency, to distinguish it from multiple carboxylase deficiency, as early reports and the majority of subsequent symptomatic patients with 3-methylcrotonylglycinuria had biotin-responsive multiple carboxylase deficiency as a consequence of deficiency of holocarboxylase synthetase (Chapter 6) or biotinidase (Chapter 7) [1, 2]. The disease was considered to be rare [3–11], until the development of programs of neonatal screening began turning up in so many patients that this

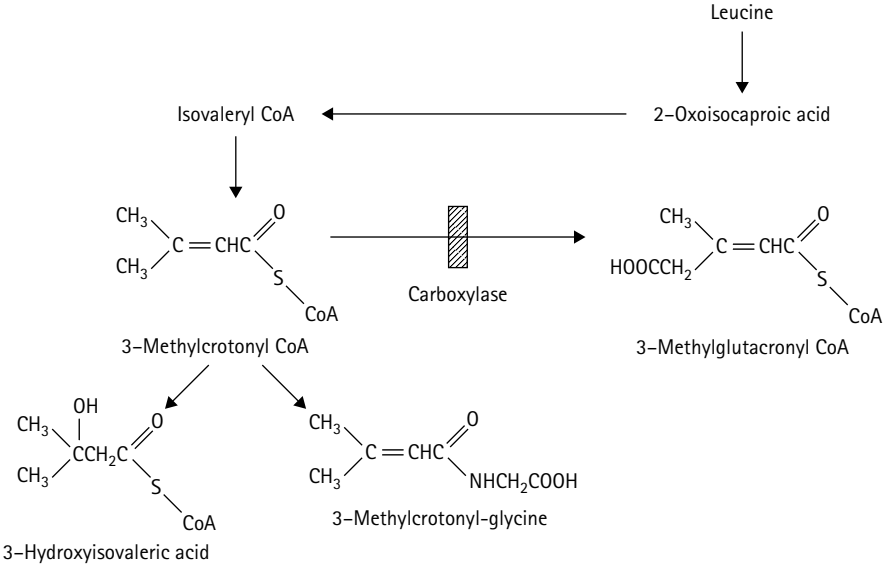

Figure 10.1 3-MethylcrotonylCoA carboxylase, the site of the defect in 3-methylcrotonylCoA carboxylase deficiency. The formation of the key metabolites results from hydration to 3-hydroxyisovaleric acid and conjugation with glycine.

disorder is being considered the most common of the organic acidemias [12]. In some instances of detection through newborn screening, it is the mother, not the newborn, who has 3-methylcrotonylCoA carboxylase deficiency. The enzyme has two (MCCa and MCCb) subunits and the A and B genes have been cloned, and mutations in each have been defined [13–16].

CLINICAL ABNORMALITIES

The classic presentation has been relatively late in infancy, between one and three years of age, with an acute episode consistent with a diagnosis of Reye syndrome [11, 17]. The episode is classic for organic acidemia in that there is massive ketosis and systemic acidosis, leading to lethargy, coma, and even a fatal outcome [9]. Hypoglycemia may be prominent, symptomatic, and life-threatening [6, 11]. Death has also occurred from cerebral edema and cardiac arrest. There may be hyperammonemia and elevated levels of transaminases in blood. There may be microvesicular and macrovesicular deposition of fat in the liver [8, 9].

The onset of the initial episode may be with vomiting or convulsions. Between episodes, vomiting is uncommon, and most patients appear completely well. Patients have noted subjectively that protein restriction led to general improvement, as well as a decrease in the number of exacerbations [3]. One patient had a neonatal onset of focal seizures and hypotonia, developed some developmental impairment and died in status epilepticus [18]. Hypotonia is commonly observed, and patients have been designated as having familial hypotonia and carnitine deficiency [17].

A number of patients have displayed quite a variety of clinical manifestations. One had chronic vomiting and failure to thrive. The onset of vomiting followed a graduation from human milk to conventional cow's milk-based formula at three weeks of life. In addition to vomiting, there was chronic diarrhea, numerous upper respiratory infections, a respiratory syncitial virus-induced bronchiolitis, and chronic mucocandidiasis. He had severe gastroesophageal reflux. Nevertheless, the existence of so many previously undiagnosed adults with the disease suggests that the general prognosis is good. In addition, most patients, once over the initial episode, have remained well and have been intellectually normal (Figure 10.2) [5, 19].

An increasing number of patients have been asymptomatic or very mildly symptomatic. Initially, these were patients diagnosed because they were siblings of patients, and many never expressed symptoms of the disease [20, 21]. A sizeable number of recent patients have been detected through newborn screening. These include infants with 3-methylcrotonylCoA carboxylase deficiency and adults discovered because their normal newly born infants failed the neonatal screening test for 3-hydroxyisovalerylcarnitine [12–14]. Some of these women have had myopathy or weakness, and carnitine deficiency, which could have been responsible for this symptomology [12]. Some also had elevated levels of uric acid and transaminases in the blood and histologic evidence of lipid deposits in the liver. These observations suggest that some of the nonspecific manifestations in earlier patients may have been unrelated to the underlying metabolic disorder. Nevertheless, the importance of the diagnosis is that any patient, regardless of even asymptomatic status, is at risk of the development, with the stress of infection, surgery, or a high protein load, of a typical Reye-like episode, which could be life-threatening. A patient who developed feeding difficulties and failure to gain weight at 11 weeks later developed seizures, spasticity, and fatal metabolic acidosis [22]. Another patient [23] had a metabolic stroke during an episode of hypoglycemia and metabolic imbalance coincident with a febrile illness. Following diagnosis and treatment, she was stable for five years of follow up, but hemiparesis and developmental delay remained. This adds to the list of metabolic diseases in which stroke-like episodes occur (see Appendix). A patient detected by newborn screening whose parents were noncompliant with recommended management was well until 19 months, but then, following a respiratory infection, developed severe acidosis, hypoglycemia, and required intubation [24]. She was found to be carnitine deficient.

Figure 10.2 NB: A four-year-old girl with 3-methylcrotonylCoA carboxylase deficiency. Her appearance and behavior have been quite normal for age. Her height was at the 25th percentile for age and the weight just below the 5th percentile. Muscle tone was reduced. She had always been a very fussy eater and ate very little. She has remained well and functions currently as an intelligent teenager. (Illustration was kindly provided by Dr Vivian Shin and the parents of the patient.)

Evidence from the California Newborn Screening Program has clarified the various populations with 3-methylcrotonylCoA carboxylase deficiency [25]. Of almost 3×10^6 infants screened 71 were diagnosed with the disease, an incidence of 1 in 41,676. Classification

as to severity indicated that only eight met criteria for biochemically severe. Unfortunately, few had had enzyme assay or mutation identification, but severe classification meant one of three criteria: enzyme activity of <0.25% of the lower limit of normal; C5OH one confirmatory profile at >150 times the upper limit of normal; either 3-HIVA or 3-MCG values >100 times the upper limit. The vast majority of patients had no symptoms attributable to the disease and were developmentally normal. The data were not sufficient to address the question of whether or not any of the biochemically mild patients ever experienced the typical ketoacidotic metabolic decompensation. Certainly, none of those we have followed did.

GENETICS AND PATHOGENESIS

The genetics of this disorder are autosomal recessive. Prenatal diagnosis should be possible by the assay of the enzyme in amniocytes or chorionic villus material [9, 26] or the direct gas chromatography-mass spectrometry (GCMS) determination of 3-hydroxyisovaleric acid in amniotic fluid [27]. Heterozygote detection may not be reliable, but values in fibroblasts, such as 21 and 42 percent of control activity, have been found in parents [9].

The molecular defect is in 3-methylcrotonylCoA carboxylase (Figure 10.1). The diagnosis should be confirmed by the assay of the enzyme in leukocytes or cultured fibroblasts [3, 9, 24]. The other carboxylases for propionylCoA and pyruvate should also be assayed, and so should biotinidase, because the distinction from multiple carboxylase deficiency is so important. A trial of biotin may be of interest, even though responsive patients with the isolated disease are rare (*vide infra*). The amounts of residual activity in fibroblasts may range from 0.05 to 3 percent in a single family [9], and up to as much as 12 percent [3]. Lymphocyte values may be much higher – approximately 46 percent of control in a patient in whom the mean fibroblast level was 10 percent [3]. Cultivation of cells in different levels of biotin does not usually affect activity. The enzyme has been purified from bovine kidney and rat liver and is an oligomer with two protein α and β subunits, like propionylCoA carboxylase [27, 28]. Complementation studies [15] have clearly shown the presence of different A and B groups.

The genes for the α and β subunits have been cloned and sequenced independently by three different groups [13–15]. The A gene is located on chromosome 3q25-28 and has 19 exons. The B gene, on chromosome 5q12-13, has 17 exons. The genes encode proteins of 725 and 563 amino acids, respectively. A number of mutations has been defined, the majority of them missense.

Genotype–clinical phenotype correlations have been particularly elusive with this gene. Expression of missense mutations led to null or severely diminished MCC activity, while patients with these mutations varied from asymptomatic to acute neonatal presentations [29]. Some individuals with no detectable enzyme activity have been asymptomatic; yet some mutations are consistent with structural activity information on the enzyme. A missense mutation M325R led to absence of labeled biotin attached to the α subunit [14]. A missense mutation in the A gene and two in the B gene involved nonconservative substitutions of residues that are highly conserved in man, plants, and fungi. Construction of a null A gene in Aspergillus abolished the ability of this organism to grow on leucine as a sole carbon source [14].

An interesting molecular mechanism has led to dominant expression of a 3MCC gene mutation [30]. Two patients found to be heterozygous for R385S in the *MCCA* gene excreted elevated amounts of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine. One had severe neurologic sequelae of an episode of metabolic imbalance at three months; the other was found by newborn screening and was asymptomatic in infancy. Both patients had a known polymorphic variant MCCA-P464H on one allele. On the other allele, MCCA-R355S coded for a normal amount of the MCCA protein but no enzyme activity.

In cotransfection experiments, insertion of the mild-type allele into a reference MCC defective cell line restored activity to 55 percent of control, while cotransfection of wild type with R385S restored activity only to 25 percent. This dominant negative effect is assumed to represent assembly of the varied protein into the normal multimeric enzyme. The other was heterozygotic for two mutations in *MCCB* (c.569A>G and c.838G>T). Among the asymptomatic patients, one had two mutations in *MCCA* including p.Lys301AlafSx10. The other two each had two mutations in *MCCB*. The other interesting issue surrounding this mutation is that the patients have been responsive to biotin.

The accumulation of 3-methylcrotonylglycine behind the block in the carboxylase leads to the excretion of 3-hydroxyisovaleric acid and 3-methycrotonylglycine (Table 10.1). The amounts are quite variable; usually but not always [3], the levels of the former are higher than the latter. An extreme example of this situation is two patients [31] with 3-methylcrotonylCoA carboxylase deficiency in whom 3-methylcrotonylglycinuria was absent; both had 3-hydroxyisovaleric aciduria. Both had a mutation in the *MCCB* gene (V339M) and undetectable messenger from the other allele; 3-methylcrotonylCoA is, after all, the immediate precursor behind the block. These experiences raise the possibility of a missed diagnosis if only GCMS organic acid analysis is used to make the diagnosis. A plasma acylcarnitine profile and/or enzyme assay should resolve this. Varying levels of the glycine conjugate, 3-methylcrotonylglycine,

Table 10.1 Urinary excretion of the key metabolites

Metabolite	Range of excretion (mmol/mol creatinine)
3-Hydroxyisovaleric acid	100–60,000
3-Methylcrotonylglycine	70–5200

in different patients may reflect varying efficiency of glycine-N-acylase. Hydroxyisovalerylglycine has not been detected, presumably because the hydroxy acid is a poor substrate for glycine-N-acylase. Supplementation with glycine has been reported [23] not to increase the excretion of 3-methylcrotonylglycine. 3-Hydroxyisovalerylcarnitine has been identified in the urine [32] and identified as a product of leucine, and its occurrence in the blood has provided the basis for programs of neonatal screening. The identification of this carnitine ester provides evidence for the intramitochondrial orgin of 3-hydroxyisovaleric acid via crotonase catalyzed conversion from 3-methylcrotonylCoA and hydrolysis of the CoA ester. This contrasts with the microsomal origin of the compound in isovaleric acidemia in which 3-hydroxyisovalerylcarnitine is not found. It is important that 3-hydroxypropionic and methylcitric acids are not found in the urine. At the time of acute ketotic illness, 3-hydroxybutyric acid, acetoacetic acid, and dicarboxylic acids are found on organic acid analysis. 2-Oxoglutaric acid excretion may be elevated and 3-methylcrotonylglutamic acid has been found [10].

Concentrations of free carnitine in the blood may be very low, and the excretion of carnitine esters is high.

The development of tandem mass spectrometry and assay of carnitine esters of CoA containing organic acids has led to highly effective programs of expanded neonatal screening. These programs have given, for the first time, reliable data on the prevalence of 3-methylcrotonylCoA carboxylase deficiency. This may be the most frequent organic aciduria [35–39]. The incidence in the population of North Carolina was reported as one in 52,000 [33]. In Australia, incidences of one in 27,000 [34] and one in 110,000 [35] have been reported. The incidence in Bavaria was one in 30,000 [36]. This is another metabolic disease that appears to be common in the Amish-Mennonite populations of the United States [12]. In data from Bavaria, less than 10 percent of patients detected by screening and found to have mutations were found to develop symptoms [37]. In addition, it was concluded that none of the symptoms reported could clearly be attributed to deficiency in this enzyme. This appears to be incompatible with the fact that patients with this disease have been observed who have had clearcut organic acidemia presentations with ketoacidosis [24]. Interestingly, it is clear that this number is small. Nevertheless, on the basis of the Bavarian data, infants in Germany are no longer screened for this disease.

Among infants found to have an abnormal newborn screening, a certain number of infants turn out to be normal products of an asymptomatic mother with MCC deficiency. In general, the levels of C50Hcarnitine tend to be higher in this situation than when they are provided by an affected infant. Elevated $C_5$0Hcarnitine is also found in holocarboxylase synthetase (Chapter 6) and in 3-hydroxy-3-methylglutarylCoA lyase deficiency (Chapter 50), as well as in 2-oxothiolase deficiency, where the elevated 2-methyl-2-hydroxybutyrylcarnitine shares the same mass as 3-hydroxyisovaleryl carnitine.

TREATMENT

Modest restriction of the intake of protein and a modest supplement of carnitine (100 mg/kg) are adequate to prevent further evidence of disease, once the diagnosis is made, assuming compliance [24]. Generally, the protein intake prescribed has been from 1.5 to 2.0 g/kg per day [5–8, 23]. A protein-free source of calories, vitamins, and minerals, such as Profree (Ross), may be useful. Alternatively, low protein intake may be supplemented by a leucine-free medical food (Analog, Maxamaid Xleu, Ross) [37–39]. We have not generally employed these in this disease. Computer programs are available [37] to aid in the preparation of diets. Recommended intake of leucine has ranged from 60 to 100 mg/kg in infants under six months, and 30–60 mg/kg in children over seven years. Carnitine therapy should be given to restore plasma concentrations of free carnitine and to achieve maximum excretion of carnitine esters, within the range of intestinal intolerance.

The acute ketoacidotic episode is treated as in classical organic acidemia with large amounts of water and electrolyte containing bicarbonate (Chapter 1) and intravenous carnitine (300 mg/kg). If prolonged parenteral nutrition is required, formulations have been designed that exclude leucine [40]. These can be supplemented with standard parenteral solutions of amino acids, so that total restriction of any individual amino acid is not pursued for more than a few days.

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11

D-2-hydroxyglutaric (DL-2-hydroxyglutaric) aciduria

MAJOR PHENOTYPIC EXPRESSION

Developmental delay, macrocephaly, seizures, vomiting, cerebral atrophy, D-2-hydroxyglutaric aciduria and defective activity of D-2-hydroxyglutarate dehydrogenase (type 1); gain of function mutations in *IDH*$_2$ the gene for isocitrate dehydrogenase (type 2); and facial dysmorphism and epileptic encephalopathy deficiency of *SLC25A1* in combined D-2 and L2-hydroxyglutaric aciduria.

INTRODUCTION

D-2-hydroxyglutaric aciduria is an organic aciduria in which the clinical phenotypic spectrum is quite broad [1–7]. It has ranged from asymptomatic to severely affected. 2-Hydroxyglutaric aciduria is identifiable by systems of gas chromatography/mass spectrometry (GCMS) organic acid analysis of the urine, but it is critical that the optical isomeric form be determined, because D-2-hydroxyglutaric aciduria and L-2-hydroxyglutaric aciduria (Chapter 12) are quite distinct diseases. The structures of D- and L-2-hydroxyglutaric acid are shown in Figure 11.1.

In 2004, Achouri and colleagues [8] identified a D-2-hydroxyglutarate dehydrogenase (Figure 11.2) in rat liver. This mitochondrial enzyme catalyzes the conversion of D-2-hydroxyglutaric acid to 2-ketoglutaric acid (Figure 11.2). Mutation analyses by Struys and colleagues [9] led to the recognition that mutations in the dehydrogenase gene are found in patients with both mild and severe clinical disease. Kranendijk *et al.* [10] have now shown clearly that there are two populations of patients with D-2-hydroxyglutaric aciduria. They refer to type I as those patients who had defective activity of the dehydrogenase enzyme and mutations in the gene. Oddly the other group, type 2 who had neither mutations nor defective activity of the enzyme, had higher levels of D-2-hydroxylglutaric acid in body fluids. These patients have now been shown to have gain of function mutations in the gene *IDH*$_2$ for isocitrate dehydrogenase [11].

Another group of patients characterized by excretion of both D-2- and L-2-hydroxyglutaric acid in the urine have severe neonatal encephalopathy, early death and mutations in the mitochondrial gene *SLC25A1* for the citrate carrier (CIC) [12].

Mutations c.18-24dup (p.Ala 9Profs*82) and c.134C>T (p.Pro45Leu) were reported in siblings who had prominent facial dysmorphic features and lactic acidosis [13].

CLINICAL ABNORMALITIES

Developmental retardation is a common feature of these diseases. Many patients are not yet known to have type 1, type 2, or type 3. In 17 patients with the severe phenotype, manifestations were early onset: at 7 months, one [1] could not sit or roll and did not fix or follow; another [3] was cortically blind. Most of the patients classified as severe [4, 5] had little evidence of mental development. Among the patients with milder presentations, mental retardation and hypotonia were the rules, though the younger sister of one patient appeared by three years to have only speech delay, and both sisters were dysmorphic, suggesting the possibility of another etiology for the mental retardation [1].

The clinical phenotype was set out by van der Knaap and associates [4] in an international survey of 25 patients with documented D-2-hydroxyglutaric aciduria. The first symptom may be vomiting. In three patients [1, 5], it was sufficiently severe that a diagnosis of pyloric stenosis was

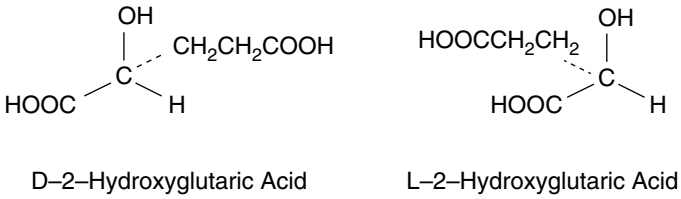

Figure 11.1 Stereochemical structures of D-2-hydroxyglutaric and L-2-hydroxyglutaric acids. The central asymmetric carbon is chiral.

made and a pyloromyotomy performed. A number of metabolic diseases, particularly organic acidemias that present in the neonatal period may be diagnosed as pyloric stenosis or similar surgical disease (see Appendix).

Macrocephaly may be another early symptom [1] (Figure 11.3). At seven months, the head circumference in one patient at 47 cm was in the fiftieth percentile for 19 months. This patient also had chronic subdural collections of fluid. Macrocephaly was also present in three of the patients classified as mild [5]; and four of the severe patients became microcephalic. Macrocephaly and subdural collections of fluid are also characteristic of glutaric aciduria resulting from glutarylCoA dehydrogenase deficiency (Chapter 9), and these diseases should be considered (Appendix) before a diagnosis of nonaccidental trauma is made.

Seizures may be grand mal or myoclonic; some were of neonatal onset, and abnormalities [1, 3] of the electroencephalogram (EEG) included hypsarrhythmia [1]. The concentration of protein in the cerebrospinal fluid (CSF) may be increased [1]. Cerebral blindness or delayed cerebral visual development has been observed [3–5].

Involuntary movements described have included chorea, dystonic posturing and episodic opisthotonic arching and extensor posturing [1, 3, 5]. Hypotonia has been observed in a number of patients [1, 3, 5], but there may also be hypertonia. Irritability and lethargy have been observed. Spasticity, increased deep tendon reflexes and positive Babinski responses have been present [1, 5].

Cardiomyopathy was found in a number of the severely affected patients, in some of whom it was clinically symptomatic. It has been dilated as well as hypertrophic. In some patients, cardiomegaly was evident only by ultrasound. One patient had a ventricular septal defect and one had a mild coarctation of the aorta and hypertrophy of the left ventricle. A patient with severe disease displayed respiratory distress and died at 10 months of cardiogenic shock as a result of cardiomyopathy [14]. Other patients have had stridor or apnea, and one required tracheostomy [4].

A variety of dysmorphic features have been noted in patients with this disease, including plagiocephaly, asymmetric ears, transverse palmar creases, epicanthal folds, a frontal upsweep of the hair and coarse features [1]. Facial dysmorphism has been particularly common, including a flat face, a broad nasal bridge and abnormalities of the external ears [15].

Spondyloenchondromatosis been reported in three patients, all products of consanguineous matings [16–18]. A pair of monozygotic twins had malar flattening and a broad nasal root [19]. One had severe developmental delay, which worsened after an infantile rotovirus infection, and she was born with esophageal atresia and a tracheoesophageal

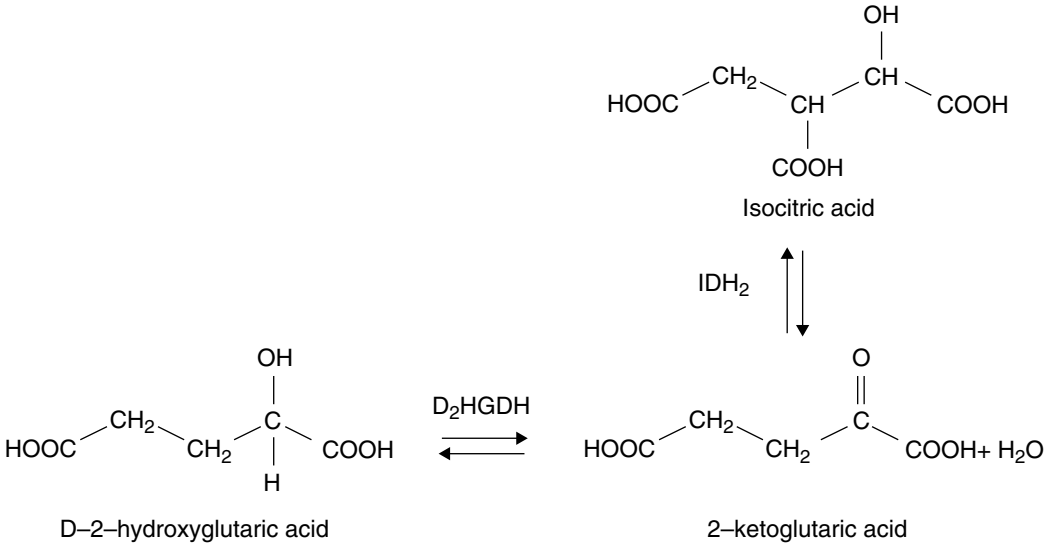

Figure 11.2 D-2-Hydroxyglutarate dehydrogenase (D-2-HGH), the molecular defect in D-2-hydroxyglutaric aciduria and isocitrate dehydrogenase (IDH_2), the newly described defect.

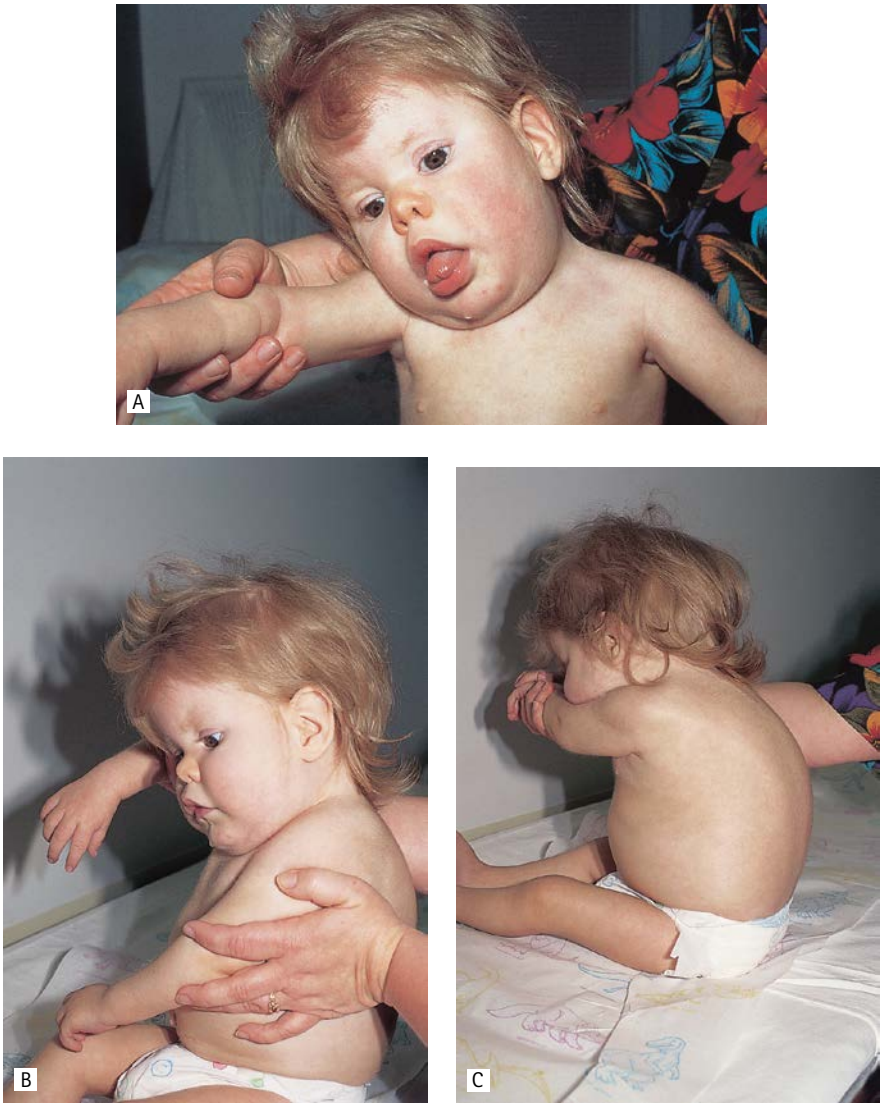

Figure 11.3 AF: A 19-month-old patient with D-2-hydroxyglutaric aciduria. (A) She had macrocephaly, deeply recessed orbits, epicanthal folds, a wide nasal bridge and an upturned nose. There were micrognathia and a carp-shaped mouth. (B) She could not sit without support. There was frontal and occipital bossing. (C) The curvature of the spine is an index of the marked hypotonia even when supported while sitting.

fistula. Her twin had macrocephaly but had normal development. A girl with D-2-hydroxyglutaric aciduria had turricephaly, brachycephaly, a broad flat face with course features and a prominent jaw [20]. Two patients with massive elevations of D-2-hydroxyglutaric acid were asymptomatic [21].

Imaging of the central nervous system with magnetic resonance (MR) or computed tomography (CT) has regularly revealed cerebral atrophy with consistent enlargement of the lateral ventricles [1, 3–5, 22–24]. White matter atrophy was progressive. Disease duration was significantly longer in patients with white matter atrophy than those without in a survey of 56 patients [22]. White matter abnormalities primarily affected the frontal and subcortical areas. Early changes were often those of a mildly swollen appearance, at least partially multifocal. The periventricular rim was relatively spared. Bilateral signal intensity abnormalities in the basal ganglia became more diffuse as cerebral white matter atrophy progressed.

Subdural effusions have been found in four patients [5]. One 14-month-old developed acute left-sided hemiparesis and a right middle cerebral artery infarction, and later a left striatal infarction, and finally infarction of the left anterior, middle and posterior cerebral arteries followed by disappearance of the left hemisphere [4]. Another patient had multiple aneurisms of the middle cerebral arteries bilaterally. A patient with severe disease who died at 10 months had, on MRI, increased T_2 signal in the substantia nigra, caudate and thalamus, lesions similar to those of patients with mitochondrial disease [13]. The infant with tracheoesophageal fistula [18] had early loss of cerebral volume, and hyperintense signal in the basal ganglia followed by dense calcification. Another infant had absence of the corpus callosum [23]. This patient also had multiple intracranial hemorrhages. Absence of the corpus callosum was also reported in two other patients with D-2-hydroxyglutaric aciduria [14, 24].

The definition of types 1 and 2 disease resulting from mutations in two distinct enzymes has permitted definition

of two different phenotypes [25]. The accumulation of D-2-hydroxyglutaric acid in body fluids has been considerably greater in type 2 patients than in type 1 (Table 11.1).

Patients with both type 1 and 2 disease have been characterized by developmental delay, hypotonia and seizures, but these manifestations have generally been milder in type 1 than type 2; three of 14 type 1 patients had no developmental delay and only four had seizures. Also, the type 2 patients were generally more severely delayed. Cardiomyopathy was only seen in patients with type 2. The course of clinical disease was often progressive in type 2, but in some it was static, and a few improved. Life expectancy in type 2 ranged from a few months to adulthood. In type 1, life expectancy is unclear.

The intriguing twins with different phenotypes [18], one with multiple congenital anomalies, severe developmental delay, and neurologic abnormalities while the other twin had an uncomplicated developmental course, despite macrocephaly, and had type 1 disease. It has been suggested [25] that postzygotic genetic changes or environmental factors could influence the outcome of type 1 disease.

The patients with skeletal dysplasia and D-2-hydroxyglutaric aciduria [16, 17], had type 2 disease and somatic mutations in *IDH1* (p.Arg132His; p.Arg143ser) [26, 27].

Patients with combined D2- and L-2 hydroxyglutaric aciduria have displayed little evidence of developmental progress [28]. Seizures resistant to anticonvulsant therapy began in the neonatal period, and death often supervened early. Macrocephaly present in one patient who lived beyond early infancy and microcephaly was found in others. Inverted nipples and abnormal distribution of fat was found in one. MRI indicated early cerebral atrophy. Another patient [12] had had agenesis of the corpus callosum and optic nerve hypoplasia and an elevated excretion of 2-hydroxyglutaric acid.

The siblings with dysmorphic facial features [28] four had hypertelorism, broad depressed nasal bridges, and micrognathia. Hypotonia was global. Each was ventilator dependent and died within two months of age. Lactate was elevated in both, up to 25 mmol/L.

GENETICS AND PATHOGENESIS

The genetic transmission of D-2-hydroxyglutarate dehydratase deficiency is autosomal recessive. Affected offspring of normal parents have been observed [1, 3, 29], as has consanguinity. Prenatal diagnosis of an affected fetus has been made by the analysis of D-2-hydroxyglutaric acid in amniotic fluid by selective ion-monitoring GCMS with stable isotope dilution internal standard [30]. In the amniotic fluid of affected fetuses, the compound was elevated 10-fold [3, 20]. In a family in which the mutation is known, analysis of the DNA may be employed in prenatal diagnosis. Genetic counseling is complicated by the occurrence of patients with normal development [18, 29].

The biochemical hallmark of these diseases is the accumulation of D-2-hydroxyglutaric acid in body fluids. The compound is readily detected and quantified by organic acid analysis of the urine. GCMS does not distinguish the enantiomers, D- and L-2-hydroxyglutaric acids (see Figure 11.1). The chiral center at the asymmetric second carbon results in differential rotation of polarized light shone on the two compounds. Light is rotated to the right by the D- (Latin dexter) form and to the left by the L- (Latin laevus) compound. The terms R and S are sometimes used for chirality D and L respectively. The identification as the D-form is accomplished by chemical ionization GCMS of the O-acetyl-di-2-butyl ester [30]. A more recently reported method [29] utilized R, R-diacetyltartaric anhydride as the agent, separated the derivatives by liquid chromatography and quantified by tandem mass spectrometry (MS/MS) [29, 31]. Urinary excretion of D-2-hydroxyglutaric acid ranged from 18 (this patient also recorded a level of 1072) to 7076 mmol/mol creatinine [1, 2, 5]. Control individuals excreted 3 to 17 mmol/mol creatinine. In our patient, the concentration in the CSF of 313 μmol/L was slightly higher than that of the plasma (283 μmol/L) [1], while in another patient [2, 3] the plasma concentration of 62 μmol/L was slightly greater than that of the CSF (25 μmol/L). Overall, the CSF level was higher than that of the plasma in all but one patient [5].

Excretion of L-2-hydroxyglutaric acid was normal in all but one patient [5]. There was no relationship between the level of D-2-hydroxyglutaric acid in urine, plasma or CSF and the severity of disease. One patient with severe disease had intermittently normal and high levels of excretion [13].

Excretion of 2-oxoglutaric acid ranged from 404 to 862 mmol/mol creatinine [1], amounts similar to those reported in 2-oxoglutaric aciduria [32]. This was found in other patients [5], and other citric acid cycle compounds were up in some, usually to a lesser level [5]. The excretion

Table 11.1 Concentrations of D-2-hydroxyglutaric acid in body fluids

	Urine mmol/mol creat. (mean; range)	Plasma μmol/L (mean; range)	Cerebral spinal fluid μmol/L (mean; range)
Type 1	969 (20); 103–2414	68 (7); 26–123	13 (3); 6–18
Type 2	2486 (19); 448–11305	366 (9); 99–757	79 (4); 30–172
Controls	6 (18); 2.8–17	0.7 (10); 0.3–0.9	0.1 (10); 0.07–0.3

of 2-oxoglutaric acid normally decreases with age, and decrease toward normal has been observed in two patients [2] while their excretion of D-2-hydroxyglutaric acid remained high. Elevated concentrations of 4-aminobutyric acid (GABA) were found in the CSF in almost all of the patients studied [5]. Levels of 20 and 28 μmol/L have been reported [1, 3]. One patient had an increased amount of glycine in the urine. Decreased levels of carnitine have been found in many patients, but many have been receiving valproate [5]. Acylcarnitine profiles have been normal, but one had multiple elevations, as seen in multiple acylCoA dehydrogenase deficiency, but without excretion of glutaric and ethylmalonic acids. Increased levels of lactic acid in the urine were found in a few patients [5, 13].

In studies of cultured fibroblasts [29, 33], the media in which cells derived from patients with D-2-hydroxyglutaric aciduria grew contained 5 to 30 times the control concentration of D-2-hydroxyglutaric acid. Studies of cultured human lymphoblasts incubated with ^{13}C-labeled glucose or ^{3}H-labeled glutamate indicated that D-2-hydroxyglutaric acid is rapidly converted to 2-oxoglutaric acid [34]. D-2-hydroxyglutaric acid is a metabolic intermediate in a variety of pathways. The simplest conversion from 2-oxoglutarate is catalyzed by D-2-hydroxyglutaric acid dehydrogenase (EC 1.1.99.6). This is the site of the defect in type 1 [35]. Mean activities in control fibroblast and lymphoblast homogenates were 208 + 207 and 1670 + 940 pmol/hour/mg protein. Cells derived from patients were less than 41 pmol/hour/mg protein.

The reaction is also catalyzed by a transhydrogenase and in the exchange 4-hydroxybutyric acid is converted to succinic semialdehyde [36]. Succinic semialdehyde is the immediate catabolic product of 4-aminobutyric (GABA), and thus, interference with this pathway and accumulation of GABA would be expected to have neurologic consequences, as in the case of GABA transaminase deficiency [37].

The molecular defect in type 1 is in the activity of D-2-hydroxyglutarate dehydrogenase (see Figure 11.2) which converts D-2-hydroxyglutarate to 2-oxoglutarate. The structure of the enzyme is homologous to that of D-lactate dehydrogenase [8] and, by analogy, it is thought to transfer its electrons to electron transfer flavoprotein (ETF). The enzyme is highly active in the liver and kidney, but it is also active in the brain and heart.

Two patients with severe disease were found to have mutations in the dehydrogenase gene [7]. One was heterozygous for two mutations in intron 1 (IVS1-23-A→G) and a missense mutation in exon 2(c.440T→G) which resulted in a substitution of serine for isoleucine (Ile 147 Ser). The intronic mutation created an alternative splice-acceptor site leading to a frameshift and premature stop. Mutations were also found in two unrelated consanguineous Palestinian families; in one of which, two affected siblings were asymptomatic; in the other, the patient had mild disease consisting of absence seizures readily controlled, difficulty reading, hyperactivity, and behavior problems [9]. In one family, the affected siblings were homozygous for an A to G transition in intron 4 (IVS4-2A→G). In the other family, the patient was homozygous for an A to G transition at nucleotide 1315 which changed asparagine 439 to aspartic acid. Expression of this mutation led to an enzyme with 13 percent of control activity. The mutation in the first family led to an unstable mRNA. In both families, each parent was heterozygous.

The twins with the very different phenotypes were compound heterozygotes for c.326-327dupTC in exon 2 and c1123G→T in exon 7 [19]. The duplication resulted in a frameshift which substituted anginine by glutamic acid at 110 and termination of the 19th codon. The missense mutation substituted tyrosine for aspartic acid 375. Each parent was heterozygous for one mutation. The unusual differences in the phenotype make genotype/phenotype correlations problematic. Twinning may have had something to do with it.

In the most recent [10] assessment, 29 mutations were found, 21 novel, each of which predicted a truncated protein. Molecular genetic abnormalities were found in all patients in whom deficiency of enzyme activity was documented. However, neither was found in over 50 percent of patients.

The key to the molecular defect in type 2 patients came from observations in cancer cells in which D-2-hydroxyglutarate accumulated in glioblastoma cells with superactivity of isocitrate dehydrogenase. Two genes IDH_1 and IDH_2 code for isocitrate dehydrogenase (see Figure 11.2). Fifteen patients were found to have gain of function mutations in IDH_2 [11]. It is recommended in the workup of patients in whom 2-hydroxyglutarate is found on organic acid analysis that the optical rotation D or L be determined first; in those with the D form, mutational analysis is carried out on D-2-HGH and IDH_2.

A lymphoblast model of IDH_2 gain of function disease has been developed [38] and employed to screen for potential inhibitors of the superactive enzyme. Oxalacetate was found to be the most effective inhibitor studied.

The mutation in glioblastomas changes the arginine R132 in the active site of the enzyme IDH_1. This mutation interferes with the normal ability of the enzyme to convert isocitrate to 2-ketoglutarate and confers a new function in the conversion of 2-ketoglutarate to D-2-hydroxyglutarate.

In 15 patients, heterozygous G to A substitution at position 419 replaced the arginine at 140 with glutamine (p.R140Q) [11]. A novel mutation at 418 (C>G) converted arginine to glycine at 140 (p.R140Q). These gains of function mutations confer an additional enzymatic capacity to convert 2-oxoglutarate to D-2-hydroxyglutarate. The greater amounts of D-2-hydroxyglutarate in type 2 than in type 1 are explained by the superactivity which overwhelms the activity of the normal dehydrogenase for D-2-hydroxyglutarate.

In eight of nine sets of parents, the mutation could not be detected, indicating a *denovo* mutation and establishing the disease as a dominant. In one family, three affected offspring of a mother with normal excretion of D-2-hydroxyglutarate suggested germ line mosaicism.

In the combined D-2 and L-2 hydroxyglutaric patients in whom the fundamental defect is in *SLC25A1*, the gene for the citrate carrier CIC. Deficiency impairs the efflux of citrate and isocitrate from mitochondria in exchange for malate. This leads to depletion of cytosolic citrate and accumulation of mitochondrial citrate. The gene is on chromosome 22q11 in the area deleted in DiGeorge syndrome. A variety of mutations was found in 12 patients, eight of whom were homozygous [12]. Missense mutations such as c.844C>T were the most common; there was one nonsense mutation, two frameshifts and a mutation which caused a splicing error. In two patients, no protein was detected on immunoblot.

The product 4-hydroxybutyric acid is also neuropharmacologically active, as illustrated by patients with 4-hydroxybutyric aciduria which is due to succinic semialdehyde dehydrogenase deficiency (Chapter 13). The elevated levels of GABA in the CSF in patients with D-2-hydroxyglutaric aciduria would be consistent with abnormalities in this pathway. Fibroblasts derived from patients with D-2-hydroxyglutaric aciduria have been found to have normal transhydrogenase activity; on the other hand, it is likely that this enzyme is responsible for the occurrence of D-2-hydroxyglutaric aciduria in patients with 4-hydroxybutyric aciduria (Chapter 13). In patients with multiple acylCoA dehydrogenase deficiency (glutaric aciduria type 2) [39] (Chapter 45), 2-hydroxyglutaric acid excretion is elevated, and it is the D-isomer that is predominant. Of course, in glutaric aciduria type 2, any hydroxyl acid accumulated might lead to the formation of D-2-hydroxyglutaric aciduria in the presence of 2-oxoglutarate in a transhydrogenase reaction.

The pathophysiology of the disease is thought to represent a developmental neurotoxicity of D-2-hydroxyglutaric acid. Incubation of pathologic concentrations of the compound with primary neuronal cultures from chickens and rats led to excitotoxic cell damage via activation of the N-methyl-D-aspartic acid receptor [40]. D-2-Hydroxyglutaric acid inhibited creatine kinase [11] in brain and in skeletal and cardiac muscle, and cytochrome c oxidase activity in fibroblasts *in vitro*, but electron transport chain activity in the fibroblasts of patients was normal. D-2-hydroxyglutaric acid was also found to inhibit *in vitro* the activity of cytochrome oxidase in rat brain fractions [11].

Patients with combined D-2 and L-2-hydroxyglutaric aciduria urine excrete increased quantities of citric acid cycle intermediates, 2-ketoglutarate, malate, fumarate and succinate.

In the siblings, there was no detectable *SLC25A1* on immunoblot study of fibroblasts [12].

In the dysmorphic siblings [28, 29], D- and L-2-hydroxyglutaric acids were elevated (D-449 mmol/mol creatinine and (L-46), although on one occurrence neither were elevated in patient two; so, the diagnosis can be missed with only one assay. Urinary excretion of citrate was low in both siblings. Elevated lactate, global hypotonia and encephalomyopathy are the clinical features found broadly in patients with mitochondrial disease.

Mutations c.18-24 dup (p.Ala9 Profs*82) and c.134C>T (p.Pro45Leu) were reported in siblings who had prominent facial dysmorphic features and lactic acidosis [28].

TREATMENT

In a patient with this disease, treatment with citrate was reported [41] to decrease the frequency and severity of seizures.

Otherwise, approaches to treatment have not been developed. The recently documented heterogeneity adds complexity. On the other hand, establishment of the molecular nature of the gene and enzyme represent major additions to the understanding of this disease. Inhibitors of the superactive enzyme in the type 2 disease are under clinical exploration.

In the combined disorder, evidence of depletion of cytosolic citrate and accumulation of citrate within mitochondria led to treatment with malate which was ineffective; in contrast, treatment with citrate (1500 mg per Kg) led to increased urinary malate and succinate and remarkable control of seizures [42].

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12

L-2-hydroxyglutaric aciduria

MAJOR PHENOTYPIC EXPRESSION

Slowly progressive ataxia, hypotonia, variable extrapyramidal and pyramidal signs, psychomotor impairment, seizures; cerebellar atrophy; rarely neonatal expression with apnea; L-2-hydroxyglutaric aciduria and deficiency of L-2-hydroxyglutarate dehydrogenase.

INTRODUCTION

L-2-hydroxyglutaric aciduria was first described by Duran and colleagues [1] in 1980 in a five-year-old Moroccan boy with psychomotor impairment. A survey of eight patients, in 1992 [2], and a later report by Barth and colleagues [3], established the usual phenotype of slowly progressive mental impairment and cerebellar signs with onset in late infancy.

L-2-hydroxyglutaric acid (Figure 12.1) is found in increased concentrations in the urine, blood, and cerebrospinal fluid (CSF). The enzyme that is defective in this disease is FAD-linked dehydrogenase that converts L-2-hydroxyglutarate to 2-oxoglutarate (Figure 12.1) [4]. The gene was mapped by Topcu *et al.* [5] by homozygosity mapping to 14q21.3. Mutations in the gene were found independently by these authors and by Rzem *et al.* [4]. About 100 different mutations have been identified [7], many of which lead to a truncated protein. The enzyme acts as a metabolite repair enzyme [6] which catalyzes the conversion of L-2-hydroxyglutarate formed in the malate dehydrogenase reaction back to 2-oxoglutarate.

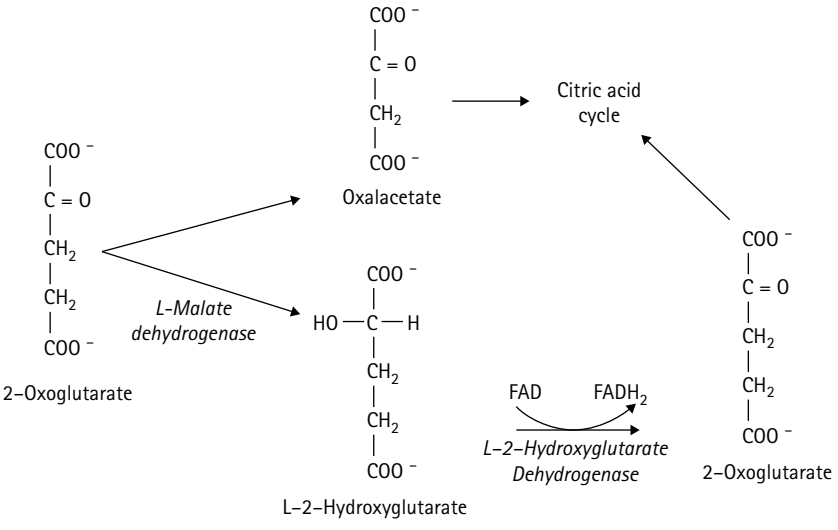

Figure 12.1 The structure of L-2 hydroxyglutaric acid, its formation and metabolite repair, through the action of L-2-hydroxyplutarate dehydrogenase, the site of the defect in L-2-hydroxyglutaric aciduria.

CLINICAL ABNORMALITIES

Patients have generally appeared well for the first year [3]. Delay in walking, abnormal gait, delay in speech, muscular hypotonia, and febrile seizures have been the presenting complaints in seven of 12 patients [3]. Patients are often macrocephalic. Progressive ataxia, variable extrapyramidal and pyramidal signs, epilepsy, and progressive mental retardation develop. In four patients, learning disability in school first called attention to the disease. In one, cerebellar signs at ten years of age were the first evidence of disease recognized. More than 100 patients are known by now [7]. Imaging of the central nervous system (CNS) has revealed abnormal loss of subcortical white matter and cerebellar atrophy [2, 3]. We reported [9] a patient with a much more severe phenotype who presented with disease that was rapidly fatal by 28 days of life.

Cerebellar manifestations were prominent in all but one of the patients summarized by Barth *et al.* [3]. Ataxia, dysarthria, and dysmetria were present. Impaired mental development was observed in all. Seizures were prominent in half of ten patients (Figure 12.2). They were either febrile or nonfebrile grand mal seizures. Spasticity has been observed [7, 8]. By adolescence, patients are usually bedridden and severely mentally retarded (IQ 40–50).

Progressive deterioration was documented in one patient [8] after a number of years of relative stability; by 16 years, she was unable to walk and had repeated seizures. Progression was also reported in two patients by Divry and colleagues [10], who emphasized ataxia, brisk tendon reflexes, and positive Babinski as extrapyramidal signs and first reported macrocephaly in both patients. Macrocephaly was also observed by Wilcken *et al.* [11] in three Australian patients of Serbian, Iranian, and Iraqi parents; one demonstrated rapid neurologic deterioration over five months and died; the others did not. One had strabismus and myopia. Two adult Japanese patients had seizures in childhood and psychomotor impairment, but began progressive degeneration after 25 years of age [12]. Nocturnal myoclonus was a feature. In addition to typical white matter disease on magnetic resonance imaging (MRI), the calvarium was thickened. Another 15-year-old boy [13] was wheelchair-bound and had impaired mental development epilepsy, optic atrophy, spastic tetraparesis, and dystonia. Six Iranian children, 4–16 years of age, had macrocephaly, ataxia, and progressive neurologic dysfunction [14].

A very different clinical picture was exemplified by a female patient who was limp at birth and had poor respiratory effort and bradycardia [9]. Initial pO_2 was 18, and Apgar scores at 1, 5, and 10 minutes were 3, 6, and 8, respectively. At 80 minutes, there was profound apnea and cyanosis requiring assisted ventilation. Episodic seizures began on day two, and the electroencephalograph (EEG) revealed a burst suppression pattern and focal epileptogenic activity. Moro, grasp, and suck reflexes were absent. She died on day 28 after the withdrawal of life support.

Imaging of the CNS (Figures 12.3, 12.4 and 12.5) in the older patients [3, 8, 13–16] by MRI or computed tomography

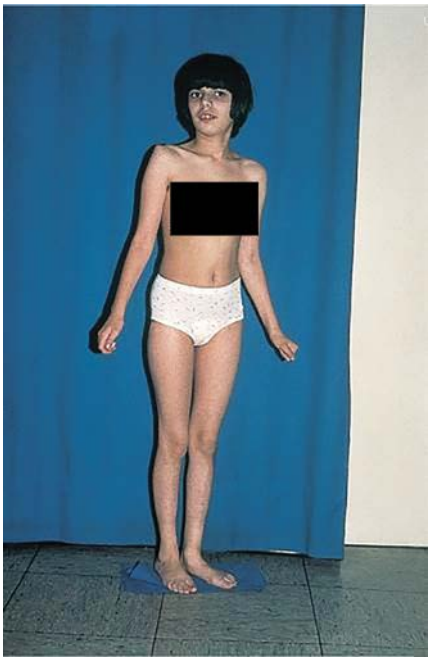

Figure 12.2 A girl with L-2-hydroxyglutaric aciduria. She was slightly delayed in her early development. At two years of age, she developed grand mal seizures and progressive ataxia became evident. By ten years of age, pyramidal signs were evident. Thereafter, the clinical picture remained stable until adolescence, when epilepsy reoccurred, and she lost the ability to walk [8].

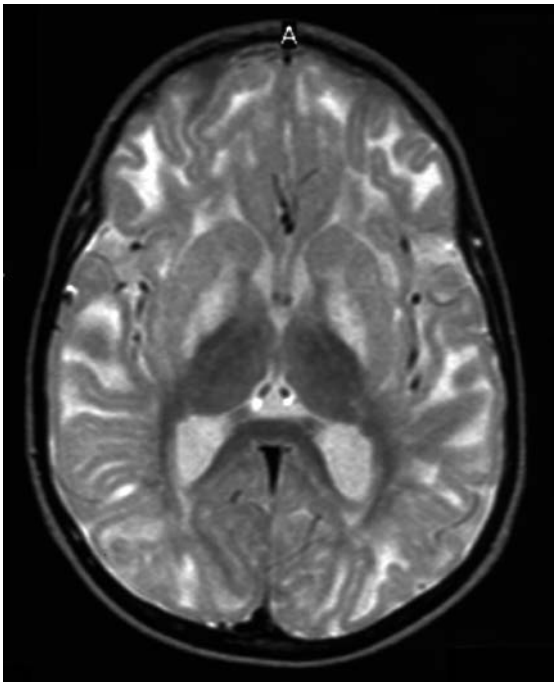

Figure 12.3 Axial T_2-weighted spin echo image of an 8½-year old boy with L-2-hydroxyglutaric aciduria. Subcortical white matter is severely deficient with much less involvement of the internal capsule and the periventricular white matter. Please note signal changes in the globi pallidi (Reproduced with permission from [15]).

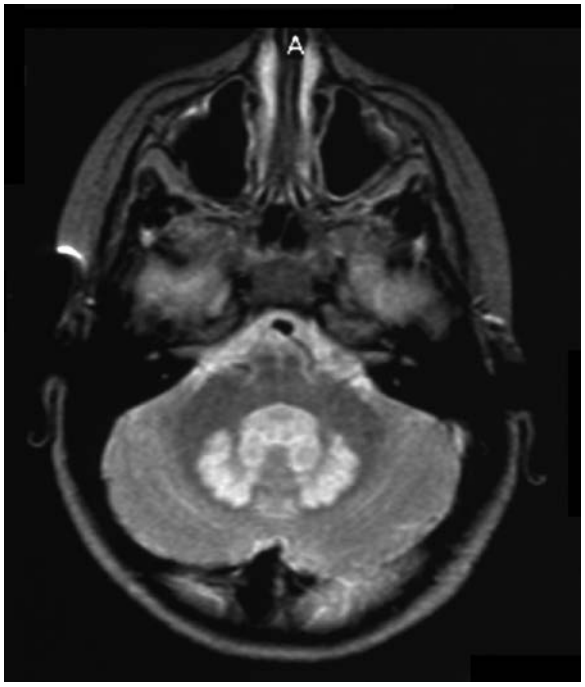

Figure 12.4 Axial T_2-weighted spin echo image of an 8½-year old boy with L-2-hydroxyglutaric aciduria. Please note hyperintense lesions in both dentate nuclei.

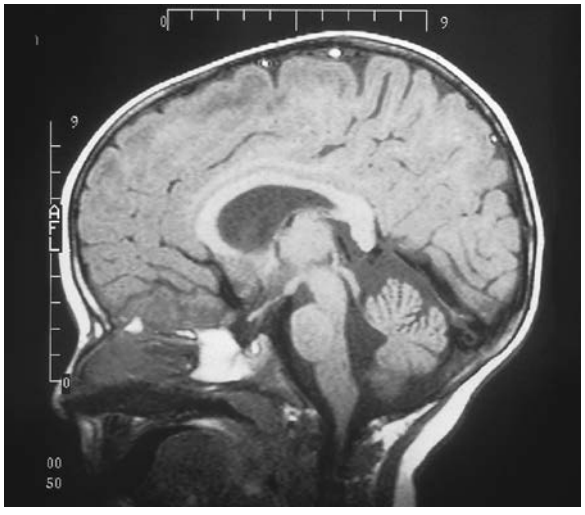

Figure 12.5 A three-year-old with L-2-hydroxyglutaric aciduria had pronounced atrophy of the cerebellum.

(CT) scan revealed mostly uniform findings comprising of a progressive loss of substance in the subcortical white matter combined with progressive cerebellar atrophy, signal changes in basal ganglia and the dentate nuclei, as well as increased ventricular size. On MRI, there was decreased signal on T_1 and increased signal on T_2 in subcortical areas. The caudate nuclei were atrophic and there were signal changes in the putamen. In the cerebellum, folial atrophy involved the vermis particularly, and there were signal changes in the dentate nuclei. Magnetic resonance spectroscopy showed abnormalities in the gray, as well as white matter, indicating neuronal loss and neurodegeneration. The pattern on neuroimaging has been stated to be unique among neurodegenerative disorders [3]. A parasellar arachnoid cyst was observed in one patient [10]. In the infant with the rapidly fatal presentation, CT scan at one day of age revealed hypodense cerebellar white matter [9]. By two weeks, this had become more hypodense, and the cerebellum was small. In one patient, calcifications were observed in the frontal lobe [17].

A brain tumor (an ependymoma) was found in a 17-year-old boy with L-2-hydroxyglutaric aciduria, and seven other patients have been identified with brain tumors, suggesting an increased risk [18, 19]. The observed malignancies varied in type and included astrocytoma, primitive neuroectodermal tumors, medulloblastoma, and oligodendroglioma. Localization was supratentorial in all cases. Diffuse gliomatosis cerebri affecting both cerebral hemispheres and upper brainstem was reported in an adult [19].

Neuropathology was reported in the infant who died at 28 days [9]. The brain stem and cerebellum were disproportionately small. The most striking changes were in the neocerebellum. The folia were small and illustrated patchy dropout of Purkinje cells. There was striking astrocytosis of the white matter in an olivopontocerebellar distribution. In a 15-year-old boy [20], cortical neuronal loss was accompanied by intense gliosis and spongiosis and vacuolation in the subcortical white similar to that found in Canavan disease.

GENETICS AND PATHOGENESIS

The disease is autosomal recessive in transmission [3]. Many families have had more than one affected offspring, and males and females have been similarly affected. A number of families have been consanguineous [3, 7, 10, 11].

Organic analysis of the urine is the usual method of detection, although analysis of the CSF [17, 21] can also serve in case finding. The concentration in the CSF was greater than that of the plasma in most patients investigated [9, 17]. Gas chromatography-mass spectrometry (GCMS) reveals a large quantity of 2-hydroxyglutaric acid. It is essential to determine the optical configuration of the compound identified because D-2-hydroxyglutaric aciduria (Chapter 11) is a different disease. A stable isotope dilution, internal standard, selected ion monitoring; GCMS method has been developed [17] in which the D- and L-acids are separated as the O-acetyl-di-2-butyl esters. In 13 patients, the concentrations of L-2-hydroxyglutaric acid in the urine were 1283 ± 676 mmol/mol creatine (range, 332–2742). In control subjects, the range was 1.3–19. In some patients, the CSF concentrations may be greater than that of the plasma [3]. The CSF concentration was 62 ± 30 mmol/L (range, 34–100), while that of the plasma was 47 ± 13 mmol/L (range, 27–62). The control ranges were 0.3–2.3 and 0.5–10 mmol, respectively [3, 17].

Prenatal diagnosis is feasible using this method. The normal amniotic fluid concentration of L-2-hydroxyglutaric acid is 3.1–5.2 mmol/L [17].

Elevated concentrations of lysine have been observed in plasma and CSF [3, 6] or only in CSF [10, 17], but loading with L-lysine did not increase excretion of L-2-hydroxyglutaric acid. In the infant with rapidly fatal disease, concentrations of lysine were normal. 2-Oxoglutarate is also used for the first step of mitochondrial lysine oxidation, i.e. the formation of saccharopine, which could explain the elevated concentrations of lysine.

Abnormalities in concentrations of carnitine or dicarboxylic acids have not been found [3]. Levels of pipecolic acid were normal [3]. In addition, a number of hydroxydicarboxylic acids (glycolate, glycerate, 2,4-dihydroxybutyrate, citrate isocitrate and putatively 2,4-hydroxyglutaric acid) were probably only found elevated in CSF because of intracerebral generation of 2-hydroxyglutaric and 2,4-hydroxyglutaric acids and trapping of other dicarboxylic acids sharing the same transporter [21].

The molecular nature of this disease remained elusive until 2004 [4–6]. L-2-Hydroxyglutarate was not a known intermediate in any eukaryotic metabolic pathway. A radiochemical assay was utilized to search for an enzyme with high affinity acting on a compound that is normally not found in mammalian tissues, and tritium was released from a racemic DL-2-hydroxy [2-^{3}H] glutarate. Ion exchange chromatography separated two enzymes, D-2-hydroxyglutarate dehydrogenase and L-2-hydroxyglutarate dehydrogenase. The latter enzyme is stimulated by FAD and active in mitochondria of many tissues. It could not be purified. Search of databases for a FAD-linked dehydrogenase acting on L-2-hydroxyglutarate yielded a bacterial enzyme that oxidizes L-malate. An homology search using the bacterial sequence identified a human protein (C14orf160) which had a mitochondrial targeting peptide. The gene which has been named *L2HGDH* is located on chromosome 14q21.3 and has ten exons and more than 75 Kb [4]. Homozygous mutations were found in three unrelated consanguineous families. Two of them, Lys71Glu and Glu176Asp, replaced highly conserved residues, and the third deleted an entire exon. It was concluded that this gene was *L2HGDH*.

Independently, Topcu and colleagues [5] used a genome-wide scan for alterations in five consanguineous families. They localized the gene to 5.4 Mb on chromosome 14q22.1. In a narrowed area of ten genes, they found mutations in patients with the disease. They named the gene *duranin* after the author of the first paper on the disease [1]. Two Turkish families were homozygous for a mutation in exon 7 which led to P302L. In two others, a deletion at c1115 in exon 9 indicated a premature stop. In another, a transversion in intron 7 yielded aberrant splicing of exon 7. Mutations have also been reported by Vilarinho *et al.* [22], often leading to a premature stop codon, an altered reading frame, or modified splicing that would yield a truncated protein. Missense mutations changed strictly conserved or semiconserved amino acid residues with very different size or polarity. A multicenter study revealed mutations in this gene in 106 patients from 83 families [7].

Incubation of lymphoblasts of patients with [$^{13}C_6$] glucose and [$^{2}H_5$] glutamic acid indicated that L-2-hydroxyglutarate is made from 2-oxoglutarate in mitochondria [23] in a reaction catalyzed by malate dehydrogenase. This has led to the conceptualization that L-2-hydroxyglutarate is formed only because L-malate dehydrogenase is nonspecific. The *E. coli* enzyme is even less specific. L-malate dehydrogenase thus slowly catalyzes the reduction of 2-oxoglutarate, the structural homologue of oxaloacetate, to L-2-hydroxyglutarate. Like D-2-hydroxyglutaric acid, L-2-hydroxyglutaric is a waste product of the tricyclic acid cycle without metabolic function, and accumulation of these acids has toxic effects. Thus L-2-hydroxyglutarate dehydrogenase catalyzes a reaction of "metabolic repair" whose purpose is to regenerate 2-oxglutarate. It reminds us that enzymes are not perfect catalysts, and metabolites, like DNA and some proteins, need mechanisms of repair.

TREATMENT

No specific therapy exists to date. Epilepsy can generally be controlled by standard antiepileptic medication.

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13

4-Hydroxybutyric aciduria

MAJOR PHENOTYPIC EXPRESSION

Mental impairment, ataxia, hypotonia, hyporeflexia, convulsions, hyperkinetic behavior, lethargy or sleep disorders bordering on narcolepsy, epilepsy, excretion of 4-hydroxybutyric acid in the urine, and deficiency of succinic semialdehyde dehydrogenase.

INTRODUCTION

4-Hydroxybutyric aciduria [1, 2] is a metabolic disorder that serves as a model for conditions in which the metabolic block causes the accumulation of a compound of established neuropharmacologic activity. Actually, since 4-aminobutyric acid (GABA) also accumulates in this disease, the disease is unique in the increased concentrations of two neuroactive compounds.

4-Hydroxybutyric acid was once developed as an intravenous anesthetic in order to obtain an analog of GABA, which would cross the blood–brain barrier. However, on testing in animals, it was found to produce convulsions [3, 4] and thus it never came to human trials. The first patient with 4-hydroxybutyric aciduria was described by Jakobs and colleagues [1] in 1981. Thirty-one patients in 21 families were studied by 1993 [2, 5]. By now, approximately 500 patients have been documented worldwide [6], of whom 114 are followed in a clinical registry maintained at Boston Children's Hospital and Harvard Medical School [7]. A report [8] of experience with 23 patients, emphasized the importance and difficulty of organic acid analysis in the diagnosis of this disorder.

Among disorders of GABA metabolism 4-hydroxybutyric aciduria has been more frequently encountered, probably because the key intermediate 4-hydroxybutyric acid is detectable by analysis of organic acids [2]. The fundamental defect is in the activity of the succinic semialdehyde dehydrogenase (EC 1.2.1.24) (Figure 13.1). In the reaction catalyzed by this enzyme, the product of GABA transamination is normally converted to succinic acid and hence to oxidation via the citric acid cycle [9]. 4-Hydroxybutyric acid is converted via β-oxidation into 3,4-dihydroxybutyric acid and thereafter to its keto acid, to glycolaldehyde and glycolic acid [10].

The genes for rat and human semialdehyde dehydrogenase have been cloned [11, 12]. The locus for the human gene (*ALDH5A1*) is chromosome 6p22 [12, 13]. Mutation analysis elucidated two exon-skipping mutations at consensus splice sites in four patients in two families [13]. A mutational spectrum has been documented in more than 50 patients from many unrelated families worldwide [14].

CLINICAL ABNORMALITIES

Impaired psychomotor development, hypotonia, ataxia, behavioral dysregulation, hyporeflexia, and epilepsy are the main features of patients with 4-hydroxybutyric aciduria and may be severe (Figures 13.2 and 13.3). Most have had especially delayed development of speech [15, 16]. Although the disease is considered slowly progressive or static in nature, severe phenotypes that include a degenerative clinical course and significant neurologic deficits have been described [17]. The first patient presented at 20 months of age with impaired motor development (Figure 13.4). He could not stand, walk, or speak. He had had brief convulsions between six and 12 months. He was ataxic and hypotonic, but not weak. The electroencephalograph (EEG) was diffusely abnormal, and computed tomography (CT) scan

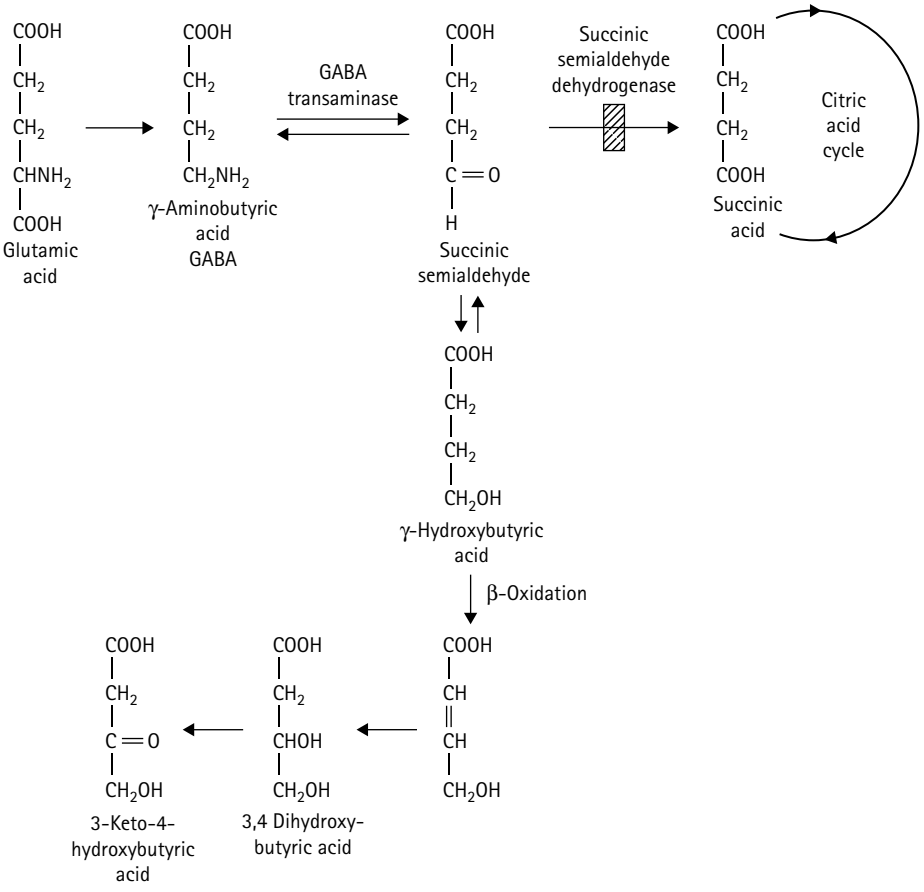

Figure 13.1 Succinic semialdehyde dehydrogenase, the site of defect in 4-hydroxybutyric aciduria, and the formation and metabolism of 4-hydroxybutyric acid.

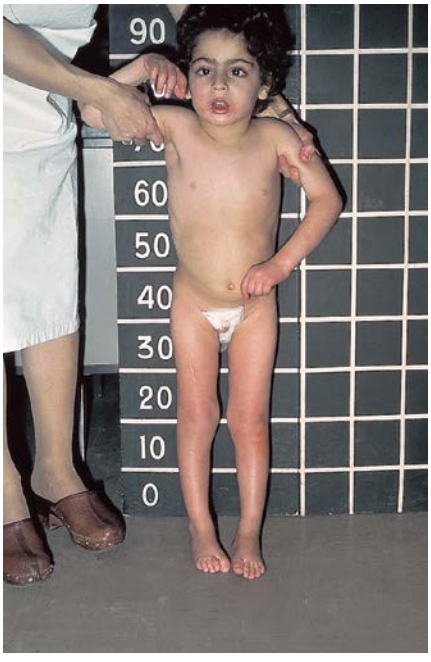

Figure 13.2 BR: A four-year-old boy with 4-hydroxybutyric aciduria. He was mentally impaired and was later admitted to an institution. (Illustration was kindly provided by Dr Priscille Divry, Hopital Debrousse, Lyon, France.)

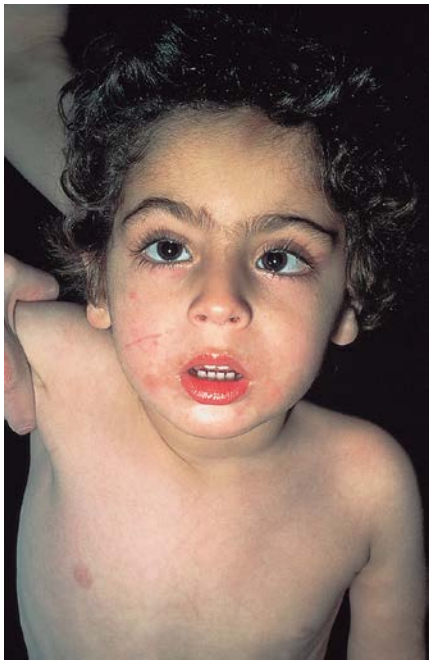

Figure 13.3 Close-up view of the patient in Figure 13.2. (Illustration kindly provided by Dr Priscille Divry, Hopital Debrousse, Lyon, France.)

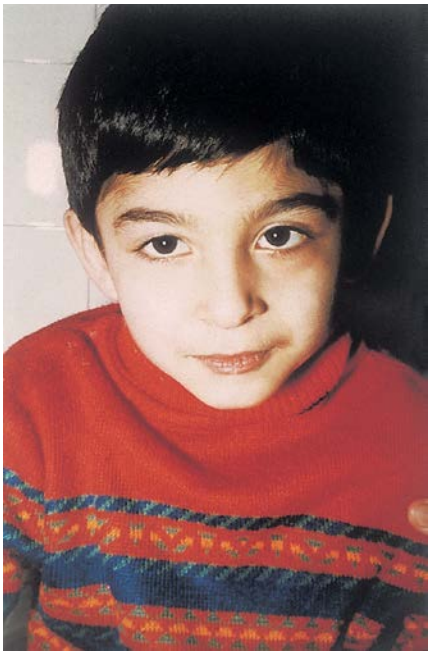

Figure 13.4 SB: The index patient with 4-hydroxybutyric aciduria (Case report). (Illustration kindly provided by Dr Dietz Rating, now of the University of Heidelberg, Germany.)

Figure 13.5 RF and RM: Lebanese siblings with 4-hydroxybutyric aciduria. (Illustration was kindly provided by Dr Dietz Rating, Heidelberg, Germany.)

revealed cerebral atrophy. Bone age was impaired. At five years of age, his condition was described as stable, without deterioration [18]. He still had no speech and an ataxic gait.

Nonprogressive ataxia and hypotonia have been recognized as characteristic of this syndrome [2, 15, 16, 18–21], along with relatively mild mental impairment. Two siblings, first seen at nine and 11 years of age, had moderate ataxia and intention tremor (Figure 13.5). Their speech was mildly dysarthric. The girl was hypotonic, but her brother was not. Deep tendon reflexes were difficult to elicit. Two years later, the ataxia in both of these children had improved considerably. Another patient had mental impairment and hypertonia and ataxia, and no improvement was noted with time. At six years of age, he

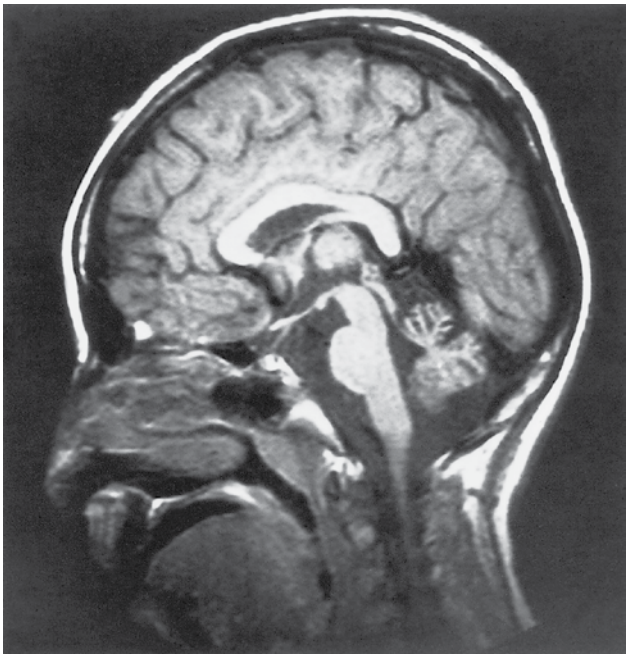

Figure 13.6 Magnetic resonance scan of the brain of a patient with 4-hydroxybutyric aciduria, illustrating cerebral atrophy.

could hardly stand and could not walk. Seizures began before one year of age. Sensory examination was normal in all of the children. Seizures occurred in slightly less than half in two groups of reported patients [7, 15, 16]. Hypotonia was observed in 60–70 percent. Optokinetic nystagmus has been described [15]. Both microcephaly and macrocephaly have been observed. One patient underwent a pyloromyotomy [15].

The clinical spectrum of the disease was expanded [8] in an assessment of 51 reported patients and a new cohort of patients with deficiency of the dehydrogenase. Mental impairment, disproportionate dysfunctioning language, hypotonia, and seizures were uniformly encountered. Only one patient presented with acute encephalopathy; she also had elevated levels of glycine [22]. Magnetic resonance imaging (MRI) of the brain may be normal [19] or may reveal cerebral atrophy (Figure 13.6). One patient had a normal MRI, followed by symmetric lesions in the globus pallidus, thalamus, and brain stem four years later [15]. Increased T_2-weighted hyperintensities in multiple regions, most commonly in the globus pallidus (43%) and dentate nucleus (17%) and occasionally in the white matter (10%) and brainstem (7%) may be more characteristic findings [8]. Seizures may be generalized tonic-clonic or myoclonic. EEG findings have included generalized spike and wave discharges in sleep, temporal focal spikes, background slowing, and occasionally photosensitivity. Ongoing problems of hyperkinesis and aggressive behavior, sleep disturbances, and hallucinations have been reported in older patients [8]. Disabling movement-induced dystonia has been described, and approximately 10 percent of patients have a more severe phenotype with

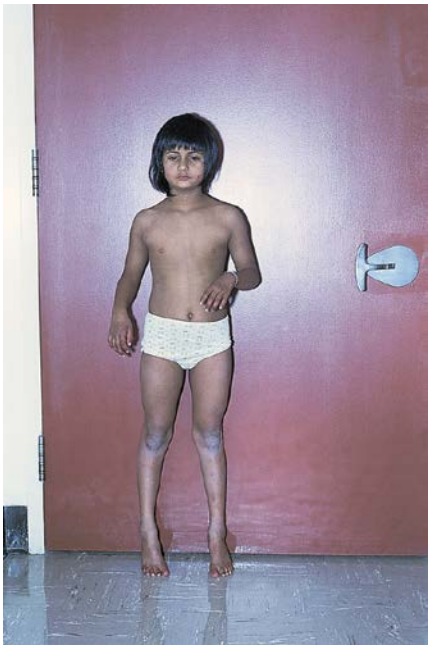

Figure 13.7 A nine-year-old girl with 4-hydroxybutyric aciduria. Her extreme hyperkinetic behavior had led to treatment with thioridazine, which ultimately led to the dyskinesia illustrated.

prominent extrapyramidal manifestations and a more progressive course. Ataxia has been observed to resolve with age [23].

Extremes of activity have been observed in different patients or families. Extremely hyperkinetic behavior has been the characteristic mode for some patients (Figure 13.7) [19, 20]. Others, such as a pair of siblings we have studied, were lethargic and somnolent to a degree that suggested narcolepsy. Sleep disturbances are common; many show excessive daytime somnolence and/or may have difficulties with initiating or maintaining sleep [24]. Some patients have been thought to be autistic [8, 19].

A possibility of two groups of phenotypes was suggested [8] by some patients whose early development was normal. Even so, ultimate disease was not mild.

GENETICS AND PATHOGENESIS

4-Hydroxybutyric aciduria is an autosomal recessive disorder. In seven of 21 families of probands, the parents were consanguineous [1, 2, 18]. Intermediate levels of enzyme activity have been found in parents [21].

Prenatal diagnosis of an affected fetus has been accomplished [25] by gas chromatography-mass spectrometry (GCMS) assay of the concentration of 4-hydroxybutyric acid in amniotic fluid. Enzyme activity was absent in fetal brain, liver, and kidney [26]. Succinic semialdehyde dehydrogenase is also measurable in chorionic villus samples [27], providing another avenue for prenatal diagnosis.

The molecular defect in 4-hydroxybutyric aciduria is in the enzyme succinic semialdehyde hydrogenase (EC 1.2.1.2) (see Figure 13.1) [5, 27, 28]. Succinic semialdehyde is the product of the transamination of GABA and is normally converted to succinic acid. When succinic semialdehyde accumulates, it is alternatively reduced to 4-hydroxybutyric acid. The enzyme is active in lymphocytes freshly isolated from peripheral blood and in cultured lymphoblasts [5, 21, 27, 28]. Accumulation of labeled succinic semialdehyde has been demonstrated in patients' lymphocytes following incubation with ^{14}C-labeled GABA, and there was no evidence of further metabolism to succinic acid [5]. Direct assay of the enzyme with ^{14}C-labeled succinic semialdehyde yielded activity that approximated 4 percent of the control level in one patient [27] and was undetectable in another. In another [20], it was as high as 21 percent in lymphocytes.

Although clinical expression can be highly variable from quite mild to severe or even fatal disease, the degree of phenotypic variation has not correlated with the amount of residual enzyme activity [2] even when monitored by a whole cell assay in which levels of activity tend to be higher [29].

The cDNA for succinic acid semialdehyde dehydrogenase has been isolated from *E. coli* [30]. Characterization of the human gene was accomplished following purification of the mammalian enzyme. Studies of the nature of mutation have begun [13, 31]. Three splicing errors have led to losses of exon 8, 9, and part of 4. In addition, an insertion, a deletion, four nonsense mutations, and a number of missense mutations have been reported [31]. The most common of over 40 detected mutations appears to be a founder mutation W204X among Europeans [14]. The second most common (R412X) was found in various locations. The most recent mutation is a homozygous missense mutation, c.901A>G (p.K301E), in *ALDH5A1*, leading to a loss of NAD+ binding with undetectable succinic semialdehyde dehydrogenase enzyme activity in lymphoblasts [32]. Among a spectrum of mutations [14], most missense mutations have led to an enzymatic phenotype of less than 5 percent of control activity in an *in vitro* expression system. Thus, residual activity is not likely to account for the very large differences in clinical phenotype.

The immediate consequence of the metabolic block is the accumulation of 4-hydroxybutyric acid. This compound has been found in large amounts in the urine in all of the studied patients [1, 2, 16]. Its nature was documented by GCMS. In the index patient [1], the amounts excreted varied from 170 to 340 mmol/mol creatinine. Concentrations in urine have ranged from two- to 800-fold the normal level [33, 34]. Succinic semialdehyde may be found in the urine, but the amounts are small. They ranged from 5 to 10 mmol/mol creatinine in one patient [1]. The ratio of 4-hydroxybutyric acid to succinic semialdehyde approximated 35 times. 3,4-Dihydroxybutyric acid has also been found regularly in the urine, and what appeared to be its 3-keto analog.

Increased concentrations of 4-hydroxybutyric acid are also found in the cerebrospinal fluid (CSF) [1, 2] and in

the plasma. In the index patient, the concentration in the CSF was 600 mm/L, approximately 60 percent of the levels found in the plasma [1]. Overall elevations have ranged from 70- to 500-fold [33, 34]. 4-Hydroxybutyric acid is not readily detected in the CSF of control individuals. Quantification of this compound, even in patients, may be spuriously low. A stable isotope dilution, internal standard method [33] has revealed consistently higher levels and would also be best for prenatal diagnosis. It has been emphasized [8] that patients may be missed in the usual methods of GCMS analysis for organic acids. The use of selective ion monitoring mass spectrometry has resulted in the diagnosis of increased numbers of patients. GABA has also been found in increased concentration in the CSF. The level of 654 pm/mL [1] was over six times the control mean.

Among patients with 4-hydroxybutyric aciduria, concentrations have been higher in younger and lower in older patients [33, 34]. This could reflect changing ratios of brain mass to body mass with age. It could provide an explanation for somnolence in young patients and hyperactivity or aggression in older patients. It has been suggested that 4-hydroxybutyric acid might bind to inhibitory sites at high concentrations and to excitatory sites at low concentrations [2].

Other compounds found in the urine of patients include dicarboxylic acids, which might suggest a disorder of fatty acid oxidation [35]. 4-Hydroxybutyric acid is, after all, a short chain fatty acid. They could result from secondary inhibition of mitochondrial fatty acid oxidation. 4,5-dihydroxyhexanoic acid identified in the urine of these patients [35] has not been found in other metabolic diseases and so may be a specific marker for this disease. It could arise from the condensation of a 2-carbon moiety with succinic semialdehyde. The occurrence of 3-hydroxypropionic acid and glycine in the urine of some patients might suggest a diagnosis of a disorder of propionate metabolism. Identification of the key compound, 4-hydroxybutyric acid, should avoid any confusion. Glycine would be a product of glycolic acid, which can be formed from β-oxidation of 4-hydroxybutyric acid [36].

TREATMENT

Treatment is symptomatic and primarily aimed at seizure management and control of the neurobehavioral manifestations. Following an initial anecdotal report of improvement in development with taurine, an open label trial did not result in significant improvement in adaptive behavioral domains [37]. Carbamazepine and lamotrigine are the only drugs which proved to be effective for seizure control. Several trials with vigabatrin (γ-vinyl-GABA), which is an irreversible inhibitor of GABA transaminase [2, 20, 38] have given inconsistent results [8]. Cerebellar signs were reported in five of six patients treated [2]. Doses employed have included 1.5 g/day in a 30-kg patient [20] in whom alertness appeared to improve and hypotonia to decrease. If attempted, patients should be closely monitored because the drug may be expected to increase levels of GABA in the central nervous system and, as indicated by GABA transaminase deficiency, this would be expected to cause neurologic disease. Several treated patients have developed seizures [39]. Valproate is an inhibitor of succinic semialdehyde dehydrogenase and it is contraindicated. Several agents were reported in the treatment of neurobehavioral symptoms including methylphenidate, thioridazine, risperidal, fluoxetine, and benzodiazepines [40]. Methylphenidate may decrease daytime somnolence.

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PART 2

DISORDERS OF AMINO ACID METABOLISM

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14

Alkaptonuria

MAJOR PHENOTYPIC EXPRESSION

Deposits of dark pigment in the sclerae, cartilage, and skin, early osteoarthritis; dark urine, homogentisic aciduria, and defective activity of homogentisic acid oxidase.

INTRODUCTION

Alkaptonuria was recognized by Garrod [1, 2] as an inborn error of metabolism, around the beginning of the 20th century. In fact, it was out of his studies of patients with alkaptonuria and their families that he conceived the idea that inborn errors of metabolism result from alterations, each in a single enzyme that is itself the consequence of a single genetic event. This was the first enunciation of what came to be known as the one-gene-one-enzyme hypothesis [3]. Alkaptonuria, or the excretion of urine which darkens on exposure to oxygen, is the result of the excretion of large amounts of homogentisic acid. The material precipitated by Boedeker [4] as the lead salt was identified by Wolkow and Baumann [5] as 2, 5-dihydroxyphenylacetic acid and named homogentisic acid, as a similar structure to gentisic acid, 2, 5-dihydroxyphenylbenzoic acid.

Alkaptonuria is usually asymptomatic for many years. Its major clinical effects are on the cartilaginous surfaces of the joints. Resulting osteoarthritis may be debilitating. The disease has been present since antiquity; evidence of the disease has been reported in an Egyptian mummy who lived around 1500 BCE [6]. Patients may also develop calcification of the aortic valves. Calculi of the urinary tract and of the prostate are relatively common.

Homogentisic acid is a normal intermediate in the catabolism of the aromatic amino acids, phenylalanine and tyrosine (Figure 14.1). It accumulates because of a defective activity of homogentisic acid oxidase [7]. This enzyme, which in mammalian systems is found only in liver and kidney, has been shown to be defective in both tissues in alkaptonuria. It catalyzes the conversion of homogentisic acid to maleylacetoacetic acid, which is ultimately converted to fumaric and acetoacetic acids. The gene for homogentisic acid oxidase has been cloned and mapped to chromosome 3q21-23 [8, 9]. The gene contains 14 exons over 60 kb of genomic DNA [10]. The spectrum of mutations has recently been summarized to include a total of 91 variants in the *HGD* gene, 62 missense, 13 splice sites, 10 frameshift, and 5 nonsense [10–12]. Most reside in exons 3, 6, 8, and 13. In the country with the greatest frequency of the disease, two variants c.16-1G>A (INV11G>A) and p.G161R, were found in more than 50 percent of patients.

A promising therapy involves treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, or NTBC (nitisinone) [13, 14]. This herbicide inhibits 4-hydroxyphenylpyruvate dioxygenase, the enzyme that produces homogentisic acid. NTBC is approved for the treatment of hepatorenal tyrosinemia (Chapter 26). It should be even more effective in alkaptonuria.

CLINICAL ABNORMALITIES

The urine of an alkaptonuric individual usually appears normal when passed. It turns dark on standing, but most people do not leave their urine standing around to be observed, so most individuals live many years, usually well into adulthood, without recognizing that they are alkaptonuric. The addition of alkali to the urine will cause the pigment to appear more rapidly (Figure 14.2) [4]. Infants have been recognized because their cloth diapers,

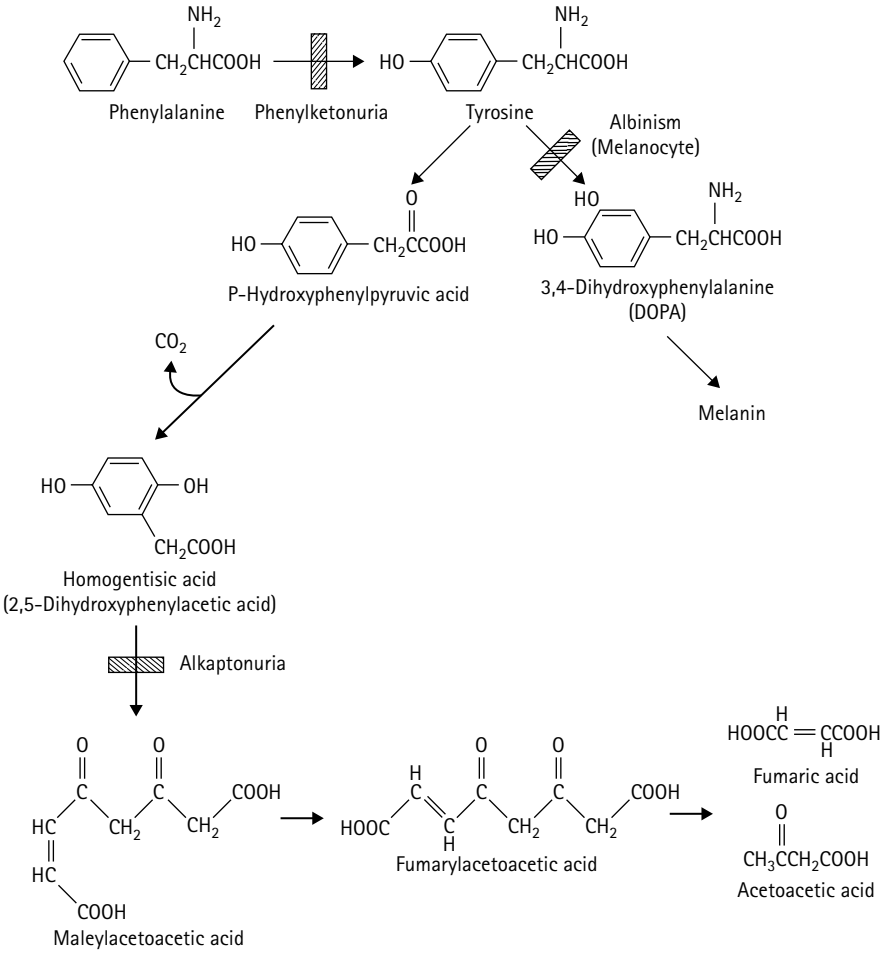

Figure 14.1 Aromatic amino acid metabolism. The site of the defect in alkaptonuria is in the homogentisic acid oxidase.

Figure 14.2 Alkaptonuria urine. The flask on the right contains fresh urine darkened somewhat; the flask on the left to which sodium hydroxide was added contains a black suspension.

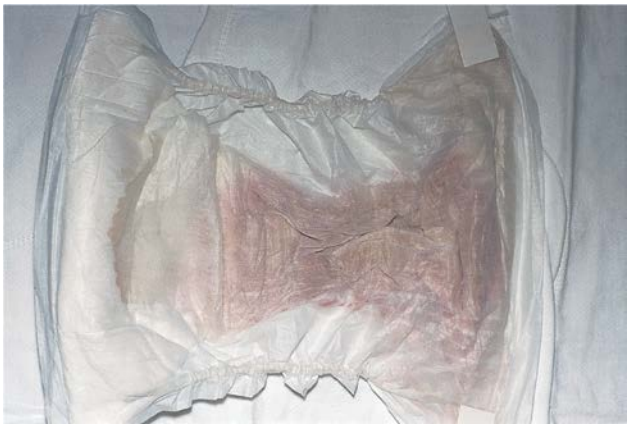

Figure 14.3 Diaper of an infant with alkaptonuria. Cloth diapers washed in detergent turn black on contact with alkaptonuric urine. We find that disposable diapers like this one become pink or red.

which had been washed with an alkaline soap or detergent, turned black or brown when they became wet with urine. Cloth diapers are seldom used today, and instead we have observed a reddish discoloration from alkaptonuric urine in disposable diapers (Figure 14.3).

In some patients, the diagnosis is suggested by a positive test for urinary-reducing substance, a feature that was also recognized in 1859 [4]. The urine does not contain glucose, and so laboratories that test urine only with glucose oxidase will miss this opportunity to find alkaptonuria. Homogentisic acid reduces the silver in a photographic emulsion, and alkaptonuric urine may be used to develop a photograph, providing a dramatic qualitative and even quantitative test

for the disease [15, 16]. Homogentisic acid may be identified by paper chromatography, and there is a specific enzymatic analysis that permits quantification [17]. We more often find it first on analysis of the urine for organic acids [18]. We also developed a high-performance liquid chromatography (HPLC) method for the quantitation of homogentisic acid and its products [19]. An adult with alkaptonuria excretes as much as 4–8 g of homogentisic acid daily [20]. The compound is excreted so efficiently that little is found in the plasma, though the amounts found by stable isotope internal standard gas chromatography mass spectrometry (GCMS) are considerably higher than those of normal plasma [21].

Patients with alkaptonuria have no symptoms as children or young adults. With age, they develop pigmentation of the sclerae or cartilage of the ear (Figures 14.4, 14.5). The condition of widespread deposition of pigment in alkaptonuria was first called ochronosis by Virchow [22] because the gray, blue, or black pigment appeared ochre under the microscope. These pigment deposits should

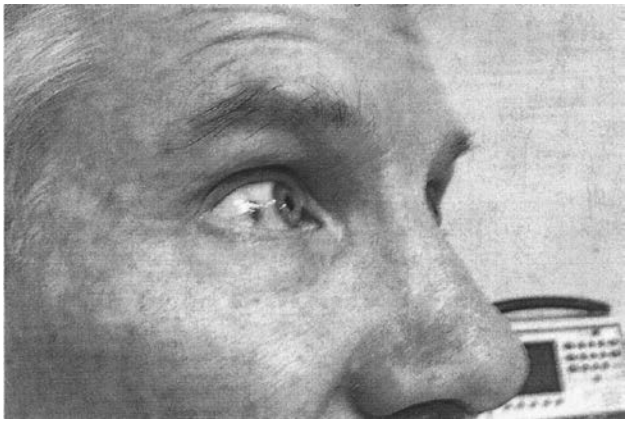

Figure 14.4 Ochronotic pigment in the sclera.

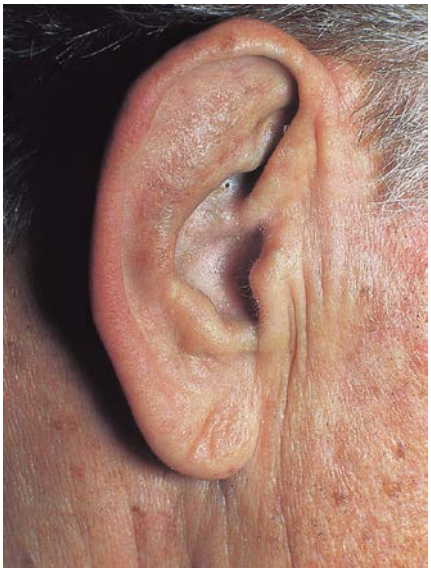

Figure 14.5 Ochronotic pigment in the cartilage of the ear.

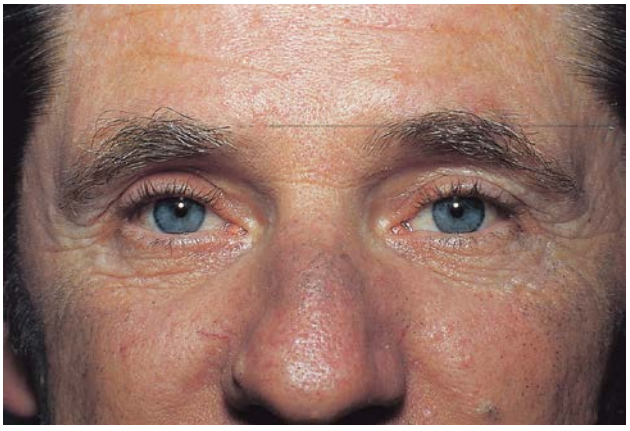

Figure 14.6 Ochronotic pigment has been deposited diffusely over the nose of this 52-year-old man.

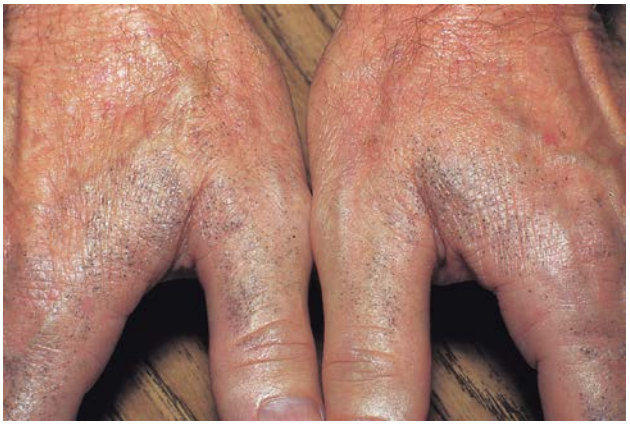

Figure 14.7 The same patient had fine, stippled pigment over the dorsum of his hands.

be visible by 30 years of-age. Actually, deposition may be widespread throughout the cartilage and fibrous tissue of the body [23–27]. Pigment may be seen at surgery and of course the diagnosis may become apparent first in this way with the rapid formation of pigment on exposure of tissues to air [28]. Pigment may be seen in the buccal mucosa and the nails. Grayish-blue longitudinal pigment was reported [29] on the finger nails, and there was pigment on the tympanic membranes. There may be deposits in the skin (Figures 14.6, 14.7), leading to areas of dusky coloration of the skin. In addition to those shown, the cheeks, forehead, axillae, and genital regions may be involved. The sweat may be dark and the cerumen brown or black.

The benign early course of these patients contrasts sharply with the severity of the ochronotic arthritis that develops early in adult life (Figure 14.8) [14, 22–24, 29–31]. The roentgenographic picture is of severe osteoarthritis (Figures 14.9, 14.10), developing much earlier than in nonalkaptonuric individuals. Some clinical features are reminiscent of rheumatoid arthritis, because there are acute periods of inflammation. Early symptoms may be in the hip or knee – large weight-bearing joints, but back pain is often

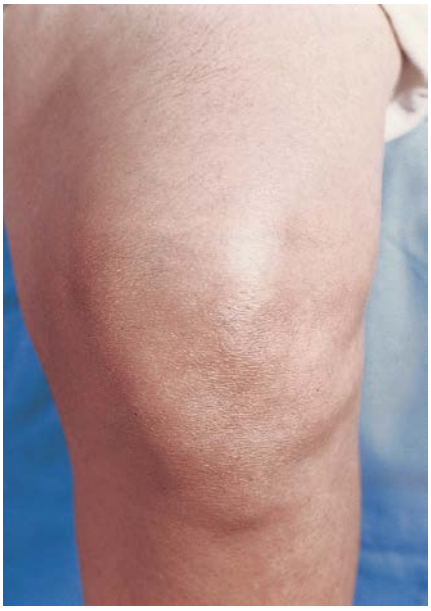

Figure 14.8 Knee of patient A.B. with ochronotic arthritis.

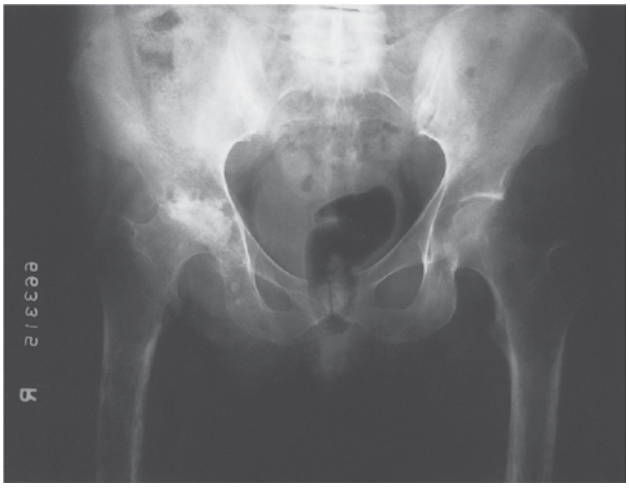

Figure 14.9 Roentgenogram of the hip illustrating the advanced, early onset osteoarthritis characteristic of this disease.

the earliest complaint [14]. Limitation of motion is seen early. Ultimately, marked limitation of motion is the rule, and ankyloses are common. The arthritis has been noted to be earlier in onset and greater in severity in males though the incidence in the two sexes is equal [32].

The roentgenographic appearance may be pathognomonic (see Figures 14.9 and 14.10) [23, 33]. The intervertebral disks undergo marked degeneration. There may be rupture of an intervertebral disc. The disc spaces become narrow and calcium is deposited. There is a variable degree of fusion of the vertebral bodies. The bamboo-like appearance (see Figure 14.10) is diagnostic of ochronotic arthritis. In contrast to rheumatoid disease, there is little osteophyte formation or calcification of the intervertebral ligaments. Mean decrement in height approximates

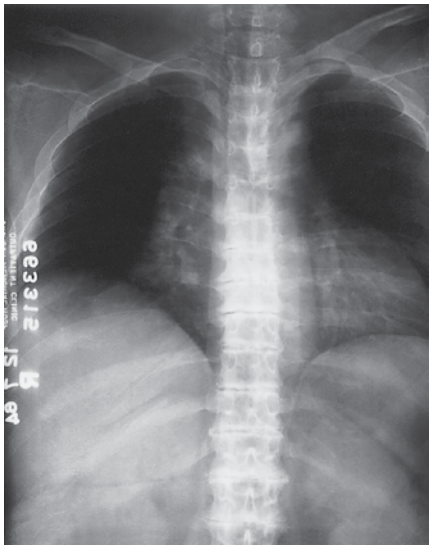

Figure 14.10 Roentgenogram of the osteoarthritic spine in the patient with alkaptonuria resembles bamboo.

8 cm [14]. In contrast to conventional osteoarthritis, the large joints at the hip and shoulder are commonly involved in ochronosis, whereas the sacroiliac joint may be uninvolved. In the involved joints there are degenerative osteoarthritic changes, occasional free intra-articular bodies [34], and calcification of the surrounding tendons. The arthritis of this disease is disabling. The patient may become bedridden or chair bound. Calcification of the ear cartilage is another roentgenographic characteristic of the disease.

Torn muscles or tendons indicate connective tissue disease [14], and thickening of the Achilles tendon is characteristic. Many have effusions of joints or the suprapatellar bursa.

Urinary tract calculi are common late findings, appearing at a mean of 64 years [14]. Urinary tract stones may also be seen in patients less than 15 years of age, and even as young as 2 years [35]. Calculi in the prostate are very common in men over 60 [14].

Patients with alkaptonuria commonly have heart disease. Mitral and aortic valvulitis may be seen at autopsy. Aortic dilation or calcifications of the aortic and mitral valve are common [14]. Myocardial infarction is a common cause of death, and there may be coronary artery calcification [14].

Index of the inflammatory nature of the disease is elevated sedimentation rate ranging from 55–110 mm/hour [14]. Levels of osteocalcin are elevated in some patients, representing the formation of new bone [14], and urinary collagen N-telopeptide, an index of bone resorption is also elevated.

GENETICS AND PATHOGENESIS

Alkaptonuria is inherited as an autosomal recessive trait [36]. Consanguinity was originally noted by Garrod [1], and subsequently by others [37]. Heterozygote detection should be possible by assay of the enzyme in the biopsied liver, but

this has not been done. Cloning of the gene for homogentisic acid oxidase in man [7, 8] has permitted molecular studies in patients with alkaptonuria and their families.

A majority of patients have represented compounds of two different mutations [10–12, 38]. In one series [14] mutations were defined in 90 percent of alleles tested. Most have been missense, but nonsense mutations and intronic mutations resulting in frameshift have been observed. Mutations have been identified in every exon but 14. There has been some clustering in exons 7 to 10 [38]. One mutation (M368V) has been observed in at least one allele in 14 patients [14]. Correlation between genotype and phenotype has not been apparent. In a highly consanguineous Turkish population, a frequent mutation was R225H [39] which have also been found in Spanish patients. A considerable heterogeneity of mutation has been observed in the populations studied [40, 41]. They are not randomly distributed, but occur in more than one third of patients in CCC repeats and in the inverted complement GGG. Hot spots for mutation in this gene are in these triplets, not in CpG dinucleotides. Three probands were found, each with three mutations, each considered to be potentially deleterious [12]. A total of 34 mutations have been reported [42], nine of them novel; these were 26 missense and four splice site mutations, two found deletions and duplications.

The disease is common in Slovakia [43]. Incidence has been estimated at 1 in 19,000. Ten different mutations have been found in this population, but two were found in more than half the chromosomes [43]. These were c183-1G>A and glycine161arginine. Mutational information has been correlated with the complex crystalline structure of the oxidase enzyme consistent with interference with folding by single nucleotide substitution [44].

Abnormality in the gene determines very low activity of homogentisic acid oxidase [17]. Defective activity of this enzyme has been documented in the autopsied kidney [45]. In normal individuals, intravenously administered ^{14}C-labeled homogentisic acid is oxidized rapidly to $^{14}CO_2$, while in alkaptonuric individuals, 90 percent is excreted unchanged in the urine [46].

The arthritis and other ochronotic elements of the disease are thought to result from the binding of highly reactive oxidation products of homogentisic acid to cartilage and other tissues. Homogentisic acid is oxidized to benzoquinoneacetic acid and ultimately to the polymeric ochronotic pigments (Figure 14.11). The reaction is catalyzed by an oxidase present in mammalian skin and cartilage [47]. Benzoquinoneacetic acid and p-quinones in general form 1, 4 addition products with sulfhydryl and amino groups [48].

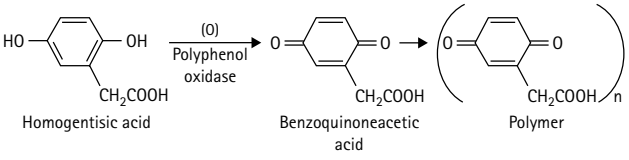

Figure 14.11 The oxidation of homogentisic acid to benzoquinoneacetic acid which precedes formation of the black polymer. Benzoquinoneacetic acid is a highly reactive compound. This or a polymeric form may bind to macromolecules in connective tissue.

TREATMENT

For many years, no treatment has been successful in reducing the accumulation of homogentisic acid or interfering with its late effects on tissues. We have shown that it is possible to reduce the formation of homogentisic acid by reducing the intake of phenylalanine and tyrosine, and this is relatively easy in an infant [15]. However, compliance with a rather difficult diet would be a major problem in this disorder in which the symptoms are so many years in the future. Dietary reduction also reduced the excretion of homogentisic acid in a 45-year-old, but the authors judged it impractical [49]. Despite these data, it has recently been stated [12] that diet has not been demonstrated to have a "straight" effect on the excretion of homogentisic acid in the urine.

Another approach was to employ reducing agents, such as ascorbic acid, in an attempt to prevent the oxidation of homogentisic acid to benzoquinoneacetic acid. Homogentisic acid inhibits the growth of cultured human articular chondrocytes. Ascorbic acid prevents this effect, and also prevents the binding of ^{14}C-homogentisic acid to connective tissues in rats [50]. We demonstrated that treatment of alkaptonuric patients with ascorbic acid was associated with a complete disappearance of benzoquinoneacetic acid from the urine. Similar results were reported by Mayatepek *et al.* [49].

Therapy with nitisinone represents an advance toward rational therapy because the compound inhibits the enzyme directly before homogentisic acid in the pathway of tyrosine catabolism (see Figure 14.1). Extensive experience in hepatorenal tyrosinemia (Chapter 22) indicated its surprising safety. Toxicity would be the reproduction of symptoms of oculocutaneous tyrosinemia (Chapter 21) and photophobia and corneal crystals have been observed in infants with hepatorenal tyrosinemia treated with NTBC [13]. In experience with two adults [14] in whom diet was not modified, 0.7 to 1.4 mg qd reduced urinary homogentisic acid excretion to 0.13 and 1.4 g per day. Plasma levels of tyrosine rose to 715 and 1288 mmol/L. There were no ocular symptoms, and slit-lamp examination was normal.

In the most recent summary of this experience, 20 patients were treated with 2 mg per day of nitisinone [51] for up to three years. There was a 95 percent reduction in urinary homogentisic acid. Nevertheless, there were no differences between treated patients and controls in such objective measurements such as range of motion at the hip. The trial was judged inconclusive. A mouse model of alkaptonuria has been developed [52]. Treatment of alkaptonuria in mice was deemed completely effective [53].

Concentrations of homogentisic in plasma were 3- to 4-fold higher than those observed in man. Pigmentation in articular cartilage was observed by 15 weeks and increased thereafter. Treatment (lifelong) with nitisinone completely prevented the deposition of pigment. This may mean that treatment should start earlier in life. Doses of nitisinone from 2 to 8 mg per day demonstrated a progressive drop in the level of homogentisic acid in the urine [54]. Changes were statistically significant only for doses 2 and 4 mg, but the numbers receiving higher doses were small. In our experience with a small number of patients, there was clear evidence of deposited pigment (53 and unpublished).

Untargeted metabolomics revealed that treatment with NTBC that produced reduction of tyrosine yielded proportional elevations of N-acetyltyrosine and γ-glutamyltyrosine [54]. Untargeted metabolomics also revealed [54] alterations in a completely separate pathway, that of the metabolism of tryptophan. Indole forms associated with the indolepyruvate pathway of tryptophan correlated directly with abnormalities in the pathway of tyrosine metabolism. It was shown that 4-hydroxyphenylpyruvate was responsible for these metabolic changes. Some of these indole products come from human cell metabolism, e.g. indole lactate/pyruvate while another, indole-6-carboxaldehyde, arose via intestinal microbial metabolism.

Arthritis, once developed, may require orthopedic treatment. In one series [14], 50 percent of patients underwent surgical replacement of at least one hip, knee, or shoulder. Mean age for joint replacement was 55 years [14]. Calcification of the aortic valve has required valve replacement in at least four patients [14, 55].

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15

Phenylketonuria

MAJOR PHENOTYPIC EXPRESSION

Mental impairment, blue eyes, blond hair, fair skin, eczematous rash, vomiting in infancy, seizures, hyperactivity, unusual odor, positive urinary ferric chloride test, hyperphenylalaninemia, and deficiency of phenylalanine hydroxylase.

INTRODUCTION

Phenylketonuria (PKU) is a disorder of aromatic amino acid metabolism in which phenylalanine cannot be converted to tyrosine (Figure 15.1). The defective enzyme, phenylalanine hydroxylase, is expressed only in liver. This disease is a model for a public health approach to the control of inherited disease since dietary treatment is effective in preventing impaired mental development. Routine neonatal screening programs have been most effective in the developed countries of the world. For these reasons, the full-blown picture of the classic disease is rarely observed today in these countries. Nevertheless, it does occur, and it is important that it be recognized.

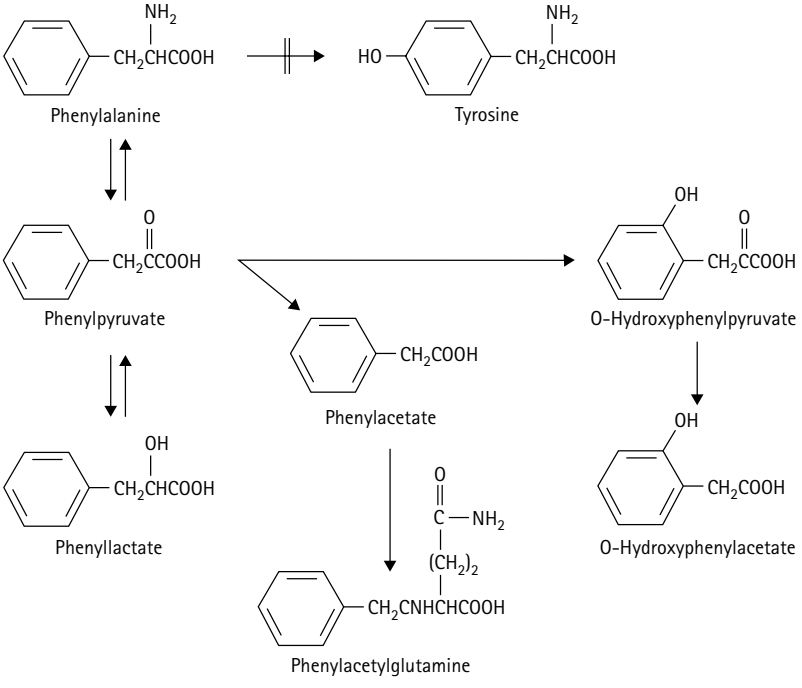

Figure 15.1 Metabolism of phenylalanine. The site of the defect in phenylketonuria is in phenylalanine hydroxylase. The compounds that accumulate as a consequence of the block are shown below.

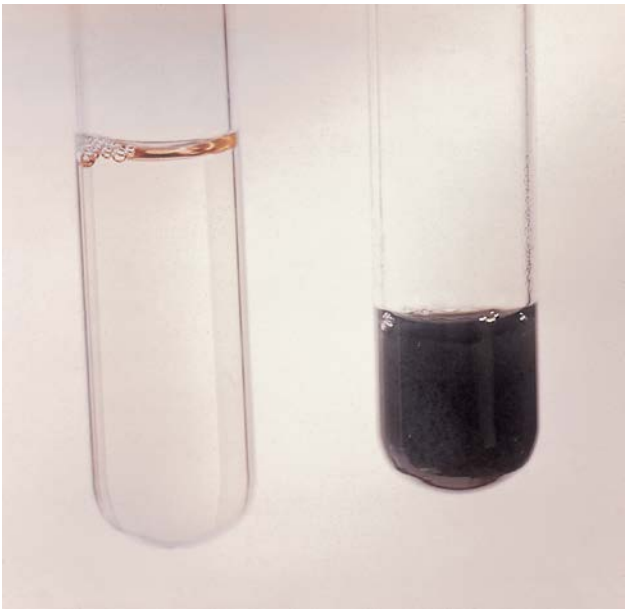

Figure 15.2 A positive ferric chloride test in a patient with untreated phenylketonuria.

The disorder was discovered by Folling [1] who tested the urine of two siblings, brought to him by their mother, by the addition of ferric chloride and noted the deep green color that results from the presence of phenylpyruvic acid (Figures 15.1 and 15.2). The term "phenylketonuria" was first proposed by Penrose [2] who recognized the disease as the first in which there was a chemical cause of mental impairment. The site of the molecular defect in the phenylalanine hydroxylase reaction was discovered by Jervis [3] who found that the conversion of phenylalanine to tyrosine could not be carried out *in vitro* by the preparations of liver obtained from patients with PKU. The gene coding for phenylalanine hydroxylase (PAH) has been identified and found to have 13 exons on chromosome 12, and a large number of mutations have been identified [4, 5]. A few mutant alleles account for the majority of human mutant chromosomes. Eight mutations have resulted in more than two-thirds of European mutant alleles.

CLINICAL ABNORMALITIES

The most important and sometimes the only manifestation of PKU is mental impairment (Figures 15.3, 15.4, 15.5, 15.6, 15.7, and 15.8). The intelligence of all but 1 percent of untreated patients is very low, with intelligence quotients (IQ) usually under 50 [6, 7]. A few patients with untreated PKU have had borderline intelligence.

Phenylketonuric infants appear normal at birth. Impaired mental development may not be evident for months. Vomiting may be a prominent early symptom. It may be severe enough to suggest a diagnosis of pyloric

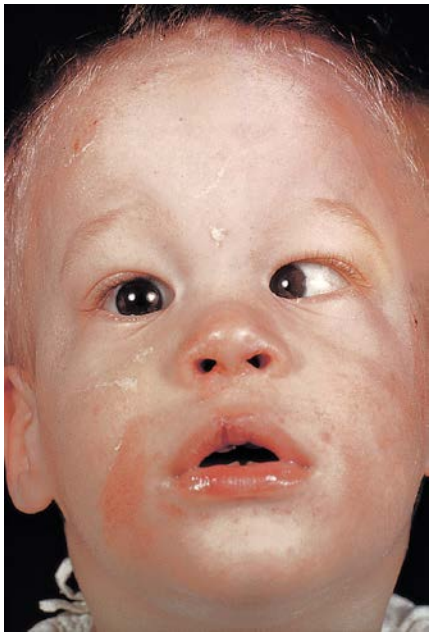

Figure 15.3 The face of this patient with phenylketonuria illustrates the rather subtle eczematoid rash. The brown eyes remind us that all patients with this disease do not have blue eyes. In addition, he had epicanthal folds and a left internal strabismus.

stenosis, and pyloromyotomy has been performed on such patients [8, 9]. Irritability, an eczematoid rash (Figure 15.3), and an unusual odor may also be observed very early in life. The odor of the phenylketonuric patient is that of phenylacetic acid (see Figure 15.1). It has variously been described as mousy, barny, wolf-like, or musty. Currently,

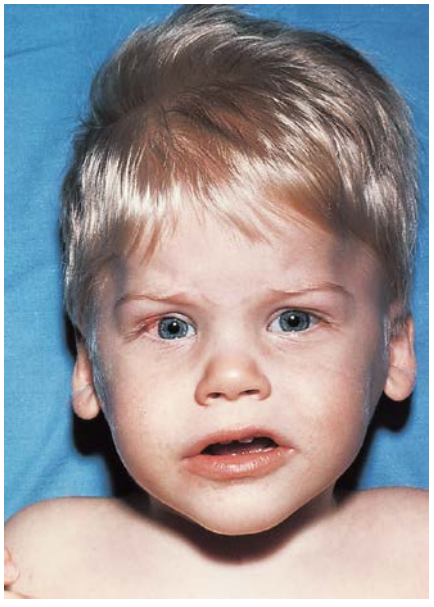

Figure 15.4 LS: This patient was diagnosed as having phenylketonuria at ten months of age. The eyes were blue, the skin fair, and the hair blond.

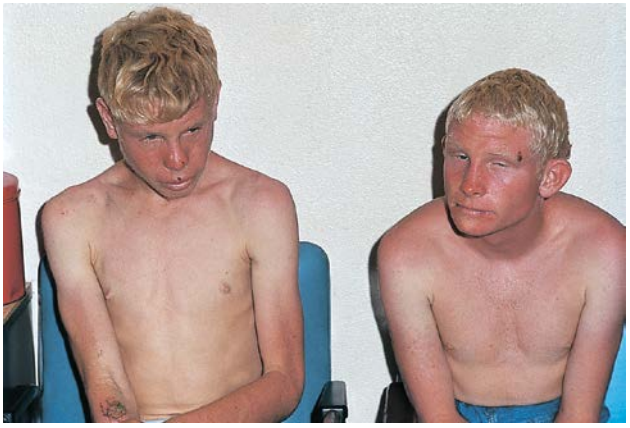

Figure 15.5 BA and LA: Severely mentally impaired, institutionalized brothers with untreated phenylketonuria. They were quite fair of hair and skin.

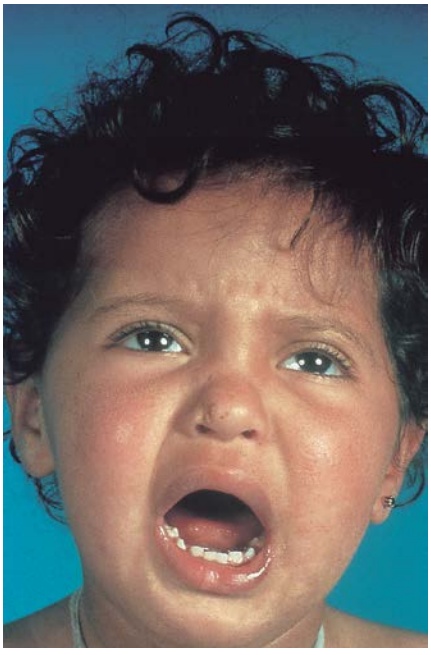

Figure 15.6 AD: A Saudi Arabian infant with classic phenylketonuria. Routine neonatal screening had not yet been initiated in that country at the time of diagnosis.

the odor is most often noted in patients with disorders of urea cycle treated with sodium phenylacetate, and, in these circumstances, it may be pervasive.

Patients with PKU are often quite good-looking children. They are fair-haired, fair-skinned, and blue-eyed in over 90 percent of the cases (Figures 15.4 and 15.5) [4]. However, there is no amount of pigment in skin, hair, or irides that excludes the diagnosis. In a family, the pigmentation of the untreated affected child is less than that of unaffected members (Figure 15.7). The dermatitis is usually mild (Figure 15.3), and it is absent in three-quarters of the patients, but it may be a bothersome symptom. Patients may complain of intractable itching in the absence of visible

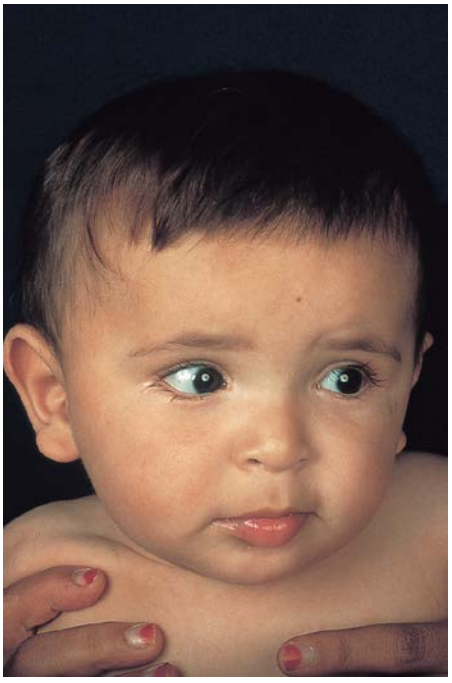

Figure 15.7 EQ: Another Saudi infant with classic phenylketonuria was considerably less pigmented than expected.

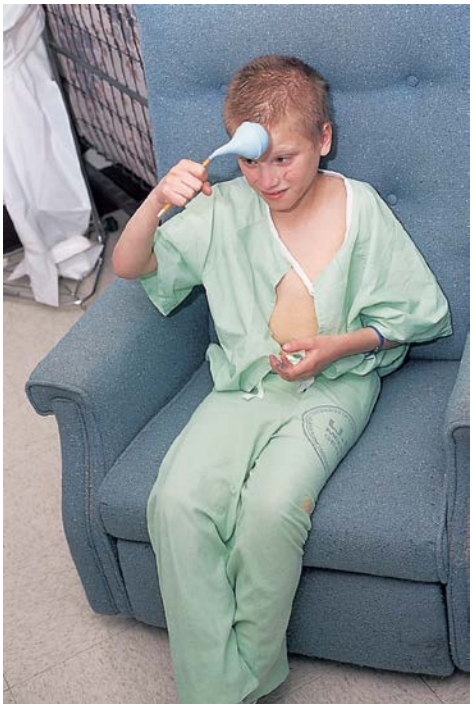

Figure 15.8 This ten-year-old boy was found in a Romanian orphanage. A diagnosis of phenylketonuria was made at the age of seven years. He had not been treated. He was very hyperactive and had seizures. His hair was blond, but his eyes hazel. He had hypertonia and had a rapid unusual limping gait, in which he leaned forward to the left, toe-walked, swinging his right arm, and keeping the left at his side.

cutaneous lesions. Sclerodermatous skin has been reported [10] in an infant with PKU.

Neurologic manifestations are not usually prominent, but about one third of the patients may have all of the signs of cerebral palsy [11]. They have spasticity and hypertonia, and have increased deep tendon reflexes. Only about five percent have these manifestations to a severe degree. They may have contractures and limitation of mobility. Hyperactivity is common (Figure 15.8), and there may be abnormalities of gait. Another one third of the patients have very mild neurologic signs, such as a unilateral Babinski response or hyperactive deep tendon reflexes. Another one third of untreated patients have no neurologic signs, except for mental impairment.

Seizures occur in about one quarter of the patients [7]. They are usually neither prominent nor difficult to manage. Nevertheless, about 80 percent have electroencephalograph (EEG) abnormalities [12]. Hyperactivity and behavior problems are common. Purposeless movements, rhythmic rocking, stereotypy, tremors, and athetosis may be seen. Somatic development tends to be normal, but stature may be short. Patients treated from the neonatal period are of normal stature. Some patients have minor malformations [13]. These include widely spaced incisor teeth, pes planus, partial syndactyly, and epicanthus (see Figure 15.3). Congenital heart disease appears to be more common in PKU than in the general population [14]. Some patients have microcephaly [11]. It has been calculated that, in the absence of treatment, a patient may lose 50 IQ points in the first year of life [15]. In the past, the majority of patients with untreated PKU required institutional care (see Figure 15.5).

GENETICS AND PATHOGENESIS

Phenylketonuria is transmitted by an autosomal recessive gene on chromosome 12q22–24.1. The gene for human PAH has been cloned. Hundreds of different alleles have been discovered, over 90 percent of which cause disease [16, 17], but only five are responsible for most human disease; the rest are rare. Almost two-thirds are missense mutations; 13 percent are small deletions; 12 percent splice-site mutations; six percent nonsense mutations; and one percent small insertions. Large deletions are rare.

Restriction enzyme polymorphism permitted heterozygote detection and prenatal diagnosis in the approximately 75 percent of families in which relevant polymorphism was identified [18]. Affected fetuses have been diagnosed prenatally in this way. Restriction fragment length polymorphism (RFLP) exists in or near the phenylalanine hydroxylase gene that permits assessment of the transmission of alleles within a family. A composite family of RFLPs on an allele is referred to as an "RFLP haplotype". Some 50 haplotypes have been described for the phenylalanine hydroxylase locus. Once the mutation in the phenylalanine hydroxylase gene is known, mutational analysis may be used for prenatal diagnosis and for heterozygote detection. This provides a practical argument for seeking the precise molecular diagnosis. In the best studied Northern European population, eight mutations have resulted in 64 percent of the mutant phenylalanine hydroxylase chromosomes [4, 5]. Two mutations, in each of which there was zero enzyme activity and cross-reacting material (CRM), accounted for 46 percent; these were an arginine to tryptophan change in amino acid residue 408 (R408W) of exon 12 and a splicing mutation of intron 12. A number of the abnormal alleles identified have involved cytosine-phosphate-guanine (CpG) dinucleotides, which are known to be highly mutable.

Expression of the mutant genes and assessment of enzyme activity *in vitro* has permitted correlations of phenotype with genotype [4]. Correlations with pretreatment concentrations of phenylalanine, and phenylalanine tolerance and the response to oral loading with protein were quite strong. The R408W mutation expressed 100 percent of mRNA, but less than one percent of enzyme activity and immunoreactive hydroxylase protein [4, 19]. Arginine to glutamine mutations in exons 5 and 7 were associated with variant phenotypes.

Different mutations have been found in other populations. Most of these have been missense mutations, such as the one that leads to complete inactivation of the enzyme in North African Jews [20]. A few deletions have been observed, such as the 22-bp deletion in exon 6 in an Arab family [21]. The primary effect of mutation in the gene is defective activity of the enzyme. This has been demonstrated by liver biopsy [22] in which activity correlated well with *in vitro* expression analysis of the mutant gene. Correlations were also excellent in eight patients tested *in vivo* with deuterated phenylalanine [23]. Analysis of genotype–phenotype correlations in an assembly of 365 patients reported [24], revealed a predominantly predictable or consistent phenotypic degree of severity in the majority. However, there were a number of genotypes that were associated with inconsistent phenotypes – both classic PKU and the variant hyperphenylalaninemia in patients with the same genotype. In a head-to-head comparison between a mutation 311C→A (A104D), associated with mild hyperphenylalaninemia, and 470G→A (R157N), with classic PKU, *in vitro* expression studies and *in vivo* ^{13}C-phenylalanine metabolism [25] indicated quite different impacts of mutation on enzyme function and physical properties. The severe mutation coded for a protein that was degraded faster than the milder variant. Although many individuals have missense mutations in the PAH gene, how such a defective enzyme will function depends on protein folding. In this regard, several studies have been published on V_{max}, K_M, and binding of its natural cofactor BH_4 [26, 27]. The results on more than 500 patients worldwide identified 60 mutant alleles. These studies clearly indicate the importance of genotyping PAH for management as well as for BH_4 treatment [27]. In 2004, Erlandsen *et al.* [28] showed that a subset of PKU patients who have a distinct

genotype showed normalization of blood phenylalanine level upon administering its cofactor BH_4. The relationship between PAH mutations and biochemical and metabolic phenotypes has also been reported [29]. The interaction of BH_4 and its impact on PAH-protein folding is of major significance in the management of hyperphenylalaninemia with certain phenotypes of PAH [30]. BH_4 increases the activity of PAH by acting as a chemical chaperone preventing protein misfolding, thus protecting PAH from inactivation [31]. Sapropterin dihydrochloride (kuvan) which is the synthetic analog of the natural cofactor, 6R-isomer of tetrahydrobiopterin, has the same activation mechanism as PAH [32].

The incidence of classic PKU has become clear from experience with the screening programs around the world. The incidence in the United States is approximately 1:10,000. Approximately 1 in 50 is a carrier of the gene. Heterozygosity has been demonstrated by assay of the enzyme in the liver, and of course, by mutational analysis.

Phenylalanine hydroxylase, the defective enzyme in PKU, has a tetrahydrobiopterin cofactor that is required for the hydroxylation of phenylalanine. In the hydroxylase reaction, a quinonoid dihydrobiopterin is formed. The reduction of this compound to reform tetrahydrobiopterin is catalyzed by dihydropteridine reductase [33, 34]. The quinonoid oxidation product is unstable and, unless it is promptly reduced, it forms the 7, 8-dihydrobiopterin and is no longer a substrate for dihydropteridine reductase, but it can be reduced by dihydrofolate reductase in the presence of the reduced form of nicotinamide-adenosine dinucleotide phosphate (NADPH). The synthesis of biopterin begins with guanosine triphosphate and proceeds through reduced neopterin (α-D-erythro-7, 8-dihydroneopterin triphosphate) to a dihydro-precursor of tetrahydrobiopterin [35, 36].

Three isozymes of phenylalanine hydroxylase have been found in the liver [37]. The three isozymes have identical molecular weights and kinetic constants, but differ in charge [37, 38]. In classic PKU, all three isozymes are missing. Immunochemical study of phenylalanine hydroxylase from a phenylketonuric human liver has revealed no CRM using antibody that reacted with normal hepatic enzyme [39]. The activity of phenylalanine hydroxylase in classic PKU has been reported as undetectable [3, 40–43].

In the presence of a defect in phenylalanine hydroxylase, the first compound that accumulates is phenylalanine itself. In classic PKU, the plasma concentration of phenylalanine is virtually always above 1200 μmol/L. It is transaminated (see Figure 15.1) to form phenylpyruvic acid, the phenylketone for which the disease was named. There is a roughly linear relationship between the concentrations of phenylalanine in the blood and the urinary excretion of phenylpyruvic acid [44]. This is the compound that is responsible for the positive ferric chloride (FeCl3) test. A deep green color is seen on the addition of 10 percent ($FeCl_3$) to the urine of patients with untreated PKU (see Figure 15.2). Phenylpyruvic acid is subsequently converted to phenyllactic acid, phenylacetic acid, and phenylacetylglutamine. Phenylpyruvate is also hydroxylated in the ortho position, ultimately yielding orthohydroxyphenylacetic acid. These are not abnormal metabolites, but normal ones that occur in abnormal amounts in PKU. It is current theory that it is this abnormal chemical milieu in which the patient with PKU lives that produces the severely impaired mental development and other manifestations of the disease.

There are a variety of secondary effects of the accumulation of phenylalanine and its metabolites. Decreased pigmentation has been related to the inhibition of tyrosinase by phenylalanine. Decreased levels of 5-hydroxytryptamine (serotonin) appear to be due to inhibition of 5-hydroxytryptophan decarboxylase by phenylpyruvic, phenyllactic, and phenylacetic acids. Decreased amounts of epinephrine, norepinephrine, and dopamine are presumably caused by inhibition of dopamine decarboxylase. The metabolites that accumulate in PKU also inhibit glutamic acid decarboxylase in brain, and this would decrease levels of 4-aminobutyric acid (GABA). Studies of protein synthesis and turnover *in vivo* via continuous infusion of ^{13}C-leucine have revealed no abnormality in PKU [45].

DIAGNOSIS

The diagnosis of PKU should be made in the neonatal period. This is accomplished by the routine screening of all infants for an elevated concentration of phenylalanine in the blood. It is generally carried out on discharge from hospital after the initiation of protein-containing feedings. A drop of blood collected from the heel on filter paper is analyzed for phenylalanine by the bacterial inhibition method developed by Guthrie and Suzi [46], or by a quantitative determination of the concentration of phenylalanine. This is now incorporated into expanded programs of screening employing tandem mass spectrometry (MS/MS). A positive screening test is usually repeated. A second positive is followed up with quantitative assay of the concentrations of phenylalanine and tyrosine in the blood confirming the phenylalaninemia and excluding transient tyrosinemia of the newborn, a common cause of a positive screening test. In the presence of an elevated concentration of phenylalanine and normal or reduced tyrosine, the patient may be admitted to hospital, where protein and phenylalanine intake are carefully monitored and fresh urine specimens collected. Patients with classical PKU ingesting a normal diet, display a very rapid rise of plasma phenylalanine to levels well over 1800 μmol/L. A concentration of 1200 μmol/L or more is diagnostic of PKU. Patients with classic PKU also excrete the metabolites phenylpyruvic acid and orthohydroxyphenylacetic acid in the urine. Cofactor abnormalities (Chapter 21) can be ruled out at that time. Precise determination of the concentration of phenylalanine in blood is of major importance. In this regard, the use of the Guthrie test is inappropriate, particularly in regions of the world where neonatal hepatitis and immature births are

Table 15.1 Protocol for phenylketonuria and variants

1. When newborn screen positive: ⟶ Obtain plasma for quantitative amino acids
   Begin low phenylalanine diet
2. Plasma phenylalanine over 340 μmol/L (6 mg/dL). Plasma phenylalanine 150–300 μmol/L (2.5–6 mg/dL)
   Repeat ↓ ↓
   Plasma phenylalanine >180 μmol/L ⟵ elevated ⟵ Repeat ⟶ Normal level <150 μmol/L
   no further control
3. Exclude cofactor deficiency
   Urinary pterins
   Dihydropteridine reductase — Abnormal ⟶ CSF BH4, neurotransmitters
   Enzyme diagnosis
   Treatment with BH4, L-DOPA
   5-OH tryptophan, carbidopa.
4. Exclude transient tyrosinemia
   Tyrosine concentrations high, exceed phenylaline ⟶ Continue to monitor concentrations and determine transient status.
   Consider ascorbic acid treatment to accelerate.
5. Phenylalanine elevated; tyrosine low.
   Phenylalanine >600 μmol/L (10 mg/dL) – Classic PKU ⟶ Diet therapy.
   Phenylalanine <300 μmol/L – Hyperphenylalaninemia ⟶ Normal diet.
   Continue to monitor phenylalanine.
   Phenylalanine 300–600 μmol/L. Hyperphenylalaninemia ⟶ Needs some dietary restriction.
6. Initial Dietary Therapy for Classic PKU means delete phenylalanine from diet as follows (phenex-1 or Lofenelac 0.7–1.0 cal/mL).

Plasma Phenylalanine		Delete Phenylalanine for:	Monitor Plasma
(μmol/L)	(mg/dL)	Hours	Quantative Phenylalanine*
240–605	(4–10)	24	qd
605–1210	(10–20)	48	qd
1210–2420	(20–40)	72	q1–3d
>2420	(>40)	96	q1–3d

*To prevent phenylalanine deficiency
When plasma phenylalanine reaches the treatment range phenylalanine is added to the diet

Individual amino acid requirements vary. The following are guidelines for initial dietary phenylalanine content dependent on the maximum pretreatment plasma levels:

Plasma Phenylalanine		Dietary Phenylalanine
(μmol/L)	(mg/dL)	mg/kg
<605	(<10)	70
605–1210	(10–20)	55
1210–1815	(20–30)	45
1815–2420	(30–40)	35
>2420	(>40)	25

Monitor neonatal levels sufficiently frequently to establish a steady state concentration at the desired level while the infant receives a constant intake of phenylalanine and tyrosine.
Aim to keep plasma phenylalanine between 100 and 300 μmol/L.

7. Monitor thereafter every week until 6 months old;
   q 2 weeks until 1 year old
   q 4 weeks until 3 years old
   q 6 months until 12 years old
   Yearly thereafter.

not rare. In such cases, tyrosinemia also occurs together with hyperphenylalaninemia (HPA). A better diagnosis for HPA is to measure simultaneously phenylalanine and tyrosine in blood. Its ratio, if higher than 3.2, indicates HPA. A good example is Taiwan where in MS/MS-based screening the most common finding was 1/5882 HPA. It is interesting to note that while classic PKU + mild PKU were 20 patients, 40 were found to have mild HPA [47]. A protocol for the management of the newborn detected by a positive screening test is given in Table 15.1.

The diagnosis of PKU is often challenged [48] with dietary phenylalanine 90–110 days after diagnosis and again after one year of age [15]. A conventional challenge in a three- to six-month-old infant is a 3-day intake of 24 oz of evaporated milk:water (1:1) which provides 180 mg/kg of phenylalanine. The challenge can be adjusted to 180 mg/kg of phenylalanine for an older, larger child. In most patients with classic PKU, the challenge yields a sharp rise in the plasma concentration of phenylalanine to 1800–2400 μmol/L in 48 hours, at which time the challenge is stopped.

It is important to remember that this challenge was developed for use with infants, and the predominant experience is at the three-month level. A dose of 180 mg/kg per day of phenylalanine for three days would be a sizeable challenge for an older child or adult with PKU. In fact, symptomatic hypoglycemia and hyperinsulinemia have been reported in a 15-year-old patient so challenged [49]. Infants in whom this test did not yield levels higher than 1200 μmol/L [42] were classified as variants. Currently, we consider those with levels over 600 μmol/L as having classic PKU (see Table 15.1).

It was the widespread screening of infant populations that led to the recognition that not all patients with HPA have classic PKU. Some variants represent molecular heterogeneity at the phenylalanine hydroxylase locus specifying variant enzymes with partial activity. Most of the variants have phenylalanine concentrations under 1200 μmol/L, and such infants can tolerate more than 75 mg/kg of phenylalanine per day. A small number of variant patients have been studied by liver biopsy [39, 42, 50], and in each a substantial defect in phenylalanine hydroxylase activity was demonstrated. Most have had levels of activity that were 10–20 percent of normal.

Transient phenylalaninemia may represent an isolated delay in the maturation of phenylalanine metabolizing enzymes. It is because of this phenomenon that patients with phenylalaninemia are routinely tested for their dietary tolerance to phenylalanine during the first year of life.

TREATMENT

The treatment of PKU is the provision of a diet sufficiently low in phenylalanine that the serum concentrations are maintained in a reasonable range and metabolites disappear from body fluids. This requires the provision of enough phenylalanine to meet the normal requirements of this essential amino acid for growth. It also requires the frequent quantitative assessment of the concentration of phenylalanine in the blood (see Table 15.1). Levels recommended as acceptable have ranged from 180 to 900 μmol/L. However, Smith and colleagues [51] have recommended a smaller window of between 120 and 300 μmol/L. Their data show a linear relationship between IQ and mean concentration during therapy over 300 μmol/L, but the differences were not clear until levels exceeded 800 μmol/L. Setting the lower level at 120 μmol/L is less secure; patients with long periods below this level appeared to have low IQ levels, only in the early cohort born prior to 1971, and no other lower limit was assessed. We strive to keep levels below 300 μmol/L and find that most patients in steady state do not approach any lower limit areas.

A patient detected in the neonatal period and managed with these guidelines should have an IQ in the normal range (Figure 15.9). The prevention of clinical disease by the restriction of dietary phenylalanine has provided the strongest evidence for the concept that the clinical manifestations of the disease result from the abnormal chemical milieu that follows the genetic defect. Preparations are now available that facilitate long-term treatment (Lofenalac-Mead-Johnson; Analog XP-Ross) and listings are available of the phenylalanine contents of foods and sources of low-protein products [52].

Sapropterin dihydrochloride (kuvan) which is the synthetic analog of the natural cofactor, 6R-isomer of tetrahydrobiopterin, is used as a chemical chaperone preventing protein misfolding thus protecting PAH from inactivation [32]. Its use is approved in the United States, European Union, and Japan. At a daily dose of 20 mg/kg, kuvan can result in increased dietary tolerance for phenylalanine or, in rare instances, replacement of the phenylalanine-restricted diet. It has the potential for the treatment of those with mild HPA who are not on diet, challenging neonates who have HPA identified by newborn screening, and the treatment of pregnant women with PKU [53, 54].

In a meta-analysis of published data, patients treated with sapropterin prior to six years of age were found to have cognitive performance no different from controls, despite ingestion of substantially more phenylalanine containing foods [55].

In the history of the management of the patient with PKU, the issue of termination of the diet has undergone

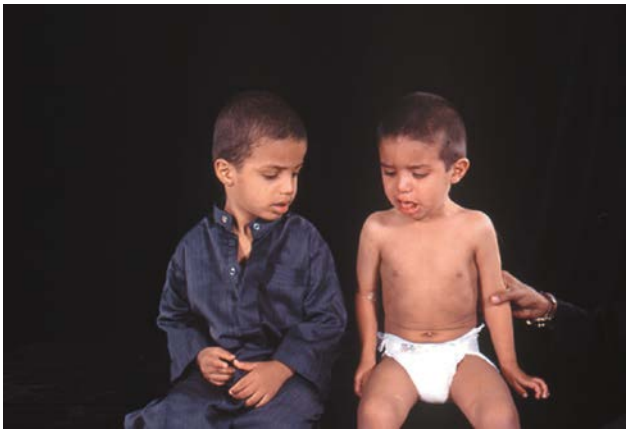

Figure 15.9 A two-year-old Saudi phenylketonuria patient who was treated as a newborn and is now in normal school is shown with his older brother who also had phenylketonuria, but was detected at one year of age and had an IQ of approximately 70. Skin and hair color were dark in both.

evolution. It was once thought that, in most patients with PKU, the diet could safely be stopped at five years of age. In a study from Poland, a decrease in IQ was found in most patients with classic PKU after discontinuing the diet [56]. Furthermore, there were difficulties in adaptation problems with performance in school and EEG abnormalities. Similarly, among 47 patients with PKU, treated at the Hospital for Sick Children in London, given a normal diet between the ages of five and 15, there was a statistically significant fall in IQ of 5–9 points after discontinuing treatment [57]. The change in IQ was uniformly negative and was progressive. Among 21 patients treated at the Universitats-Kinderklinik, Heidelberg, who were given a relaxed low phenylalanine diet rather than a normal diet at about the same time as the London group, there were smaller and nonsignificant falls in IQ. Data from the United States Collaborative Study on 115 children suggested that discontinuing dietary treatment at six years of age led to a reduction in IQ [58]. There were significant differences in school performance as measured by the Wide Range Achievement Test.

Actually, the IQ alone may not be the most sensitive criterion on which to base this decision in an individual patient. Other aspects of clinical condition which might benefit from treatment longer than necessary to produce a stable IQ might affect the way a child functions in society. Behavioral abnormalities are common in children with PKU, despite early diagnosis and treatment and normal IQ. Mannerisms, hyperactivity, and signs of anxiety have been reported in eight-year-old children treated since early neonatal diagnosis, and those whose diet was less strictly controlled were twice as likely to display abnormal behaviors than those more strictly controlled [59]. On the other hand, a study [60] of 586 German ten-year-old PKU patients via a personality questionnaire failed to reveal differences from controls. The long-term effect of treatment on intelligence of patients with PKU has been assessed in a British study of 15 well-managed individuals with PKU treated by two years of age, who had normal IQ. They were found to have defects in planning and attention domains [61]. A comparative study on 35 well-managed PKU patients as compared to 35 diabetic individuals matched in sex, age, and socioeconomic status were observed to have a reduced speed in performance [62]. It is certain that early detection and treatment prevent serious consequences of PKU. However, more and more hidden disabilities are being noticed later in life in well-treated PKU patients. These include mostly executive dysfunction (EF), mild reduction in mental processing speed, social difficulties, and difficulties in establishing interpersonal relationships and emotional problems, which may go unnoticed if not particularly looked for [63]. Children and adolescents with PKU showed lower performance in several EF skills: initiation of problem solving, concept formation, and reasoning. Performance on EF tasks requiring inhibitory control, cognitive flexibility, and set shifting decreased at higher levels of phenylalanine. Levels of phenylalanine were positively correlated to age and inversely related to dietary adherence. Therefore, there is a need to monitor EF skills in patients with PKU during the transition to, and during, adolescence.

A study in 2010 on well-treated PKU patients detected suboptimal outcomes in neurocognitive/psychosocial outcome, in nutrition/growth, quality of life, in bone and brain pathology, and in incidence of maternal PKU [64]. The exact biochemical mechanism underlying the subtle neurocognitive defects remains unknown. However, ^{18}F-deoxyglucose positron emission tomography (PET) revealed decreased glucose utilization in prefrontal cortex, somatosensory, and visual cortices, but increased activity in subcortical regions, such as the thalamus and limbic system [65]. The best management to prevent later appearance of subtle neurologic defects remains early detection and vigorous treatment.

If high levels of phenylalanine continue to impair myelinization, one might even expect treatment to be useful well up to puberty, since myelination at least in the formatio reticularis is not finished at eight years of life. In addition, the effect of high concentrations of phenylalanine on synaptogenesis is not known. Some older patients find that their skin feels better with modest restriction of phenylalanine. In any case, the rigidity with which one controls the level of phenylalanine can probably be relaxed after six years of age, but it is prudent to continue some restriction in the intake of phenylalanine. A study of 25 adults with PKU, all of whom had been treated early [66] revealed normal intelligence, but in each patient scores were lower than control siblings in measures of intelligence and attention. Patients were advised to continue dietary restriction for life, but only ten followed this regimen. Others discontinued treatment before or during adolescence. Intellectual outcome appears to have best been predicted by the presence or absence of early insult to brain, while performance on a test of problem-solving correlated best with concurrent levels of phenylalanine even in adulthood. As patients become older, some relaxation of dietary control appears inevitable. In a recent study of 95 patients treated from the neonatal period and assessed at 12 years of age, best cognition results were those of patients whose phenylalanine values were kept consistently below 900 μM.

Reduced concentrations of carnitine in serum have been found in patients with PKU [67]. This was the case in those less than two years of age managed with a restricted diet. In contrast, untreated infants with PKU had normal levels of carnitine as did older patients. The data provide an argument for supplementation with carnitine at least in infants treated for PKU.

Poor linear growth has been observed in some patients with PKU, and this has been thought to result from protein insufficiency. The level of prealbumin has been found [68] to correlate well with protein adequacy in this disease, and the threshold level is 20 mg/dL. Linear growth can be expected to be impaired in patients with levels lower than 20 mg/dL.

Among the complications of lifelong PKU and its treatment are effects on the kidney. Decrease in glomerular filtration rate, proteinuria and hypertension were found in patients 15–43 years of age [69]. There was no correlation with phenylalanine hydroxylase phenotype. The high lifelong intake of mixtures of amino acids was thought to have been nephrotoxic.

MATERNAL PKU

Elevated phenylalanine blood levels in pregnant women cause a syndrome known as "maternal PKU" (MPKU). The elevated phenylalanine has teratogenic effects on the developing fetus. The findings include global developmental delay, microcephaly, facial dysmorphism, congenital heart disease (CHD), and low birth weight [70]. These findings are dramatically reduced when maternal phenylalanine levels are well maintained between 120 and 360 μM. Other studies have also indicated that if the metabolic control is started before eight weeks of pregnancy, a better result is obtained. A British study on all MPKU between 1978 and 1997 compared head circumference, IQ at four and eight years of age, and CHD. Abnormalities were significantly lower when the treatment of the mother was initiated before eight weeks of pregnancy [71]. Education of young women with PKU and initiation of metabolic control at least eight weeks before pregnancy is essential for the prevention of MPKU [71, 72].

Male patients with PKU may have low sperm counts and semen volume. A survey of male patients over 18 years in the United States identified 40 men who had 64 children, but did not yield data on fertility rate [73]. Abnormalities were not identified in live-born offspring that could be related to paternal PKU.

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16

Hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin

MAJOR PHENOTYPIC EXPRESSION

Progressive encephalopathy characterized by severe truncal hypotonia, decreased spontaneous movements, muscular rigidity of the extremities, movement disorders, including distal chorea and dystonia, mental impairment, microcephaly, epileptic seizures, hyperphenylalaninemia (HPA), and defective synthesis of tetrahydrobiopterin (BH_4) because of defective activity of GTP cyclohydrolase (GTPCH), 6-pyruvoyltetrahydropterin synthase (PTPS) or sepiapterin reductase (SR), and defective recycling of BH_4 due to deficiency of dihydropteridine reductase (DHPR) or pterin-4a-carbinolamine dehydratase (PCD).

INTRODUCTION

The existence of variant forms of HPA resulting from abnormalities of cofactor synthesis was predicted with the discovery of biopterin (Figure 16.1) and its role in the phenylalanine hydroxylase reaction (Figure 16.2) [1–4]. BH_4 is an essential cofactor not only for phenylalanine hydroxylase, but also for tyrosine and two tryptophan hydroxylases, three nitric oxide synthases, and glyceryl-ether monooxygenase [5]. Defective activity of tyrosine and tryptophan hydroxylases is relevant to neurologic deterioration in patients with BH_4 deficiency [6]. The first

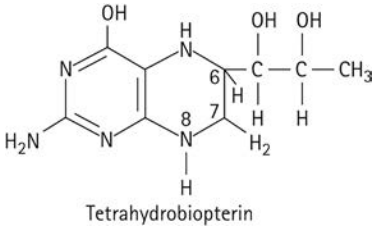

Figure 16.1 Tetrahydrobiopterin (BH_4), the pteridine cofactor of phenylalanine hydroxylase.

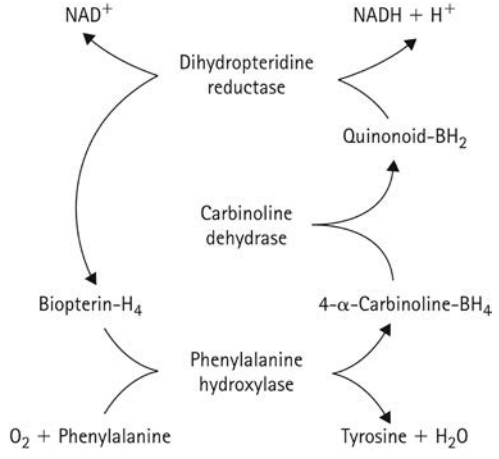

Figure 16.2 Dihydropteridine reductase and pterin-*4a*-carbinoline dehydratase, enzymes involved in regeneration of BH_4 following the phenylalanine hydroxylase reaction. Defects in this system lead to defective function of BH_4 in the metabolism of phenylalanine and the neurotransmitters. BH_4, tetrahydrobiopterin; BH_2, dihydrobiopterin; NAD1, nicotinamide dinucleotide, and NADH, its reduced form.

patients were reported in the 1970s as an outgrowth of the programs of newborn screening for phenylketonuria (PKU). A majority of the patients recognized were diagnosed because they developed progressive cerebral deterioration despite an early neonatal diagnosis of HPA and effective dietary control of the levels of phenylalanine in blood.

Patients are now being diagnosed earlier because of the initiation of programs in which all hyperphenyalaninemic infants are being investigated for the possibility of defective metabolism of biopterin. However, it has been documented that it is possible to miss a patient with abnormal synthesis of BH_4 because early phenylalanine levels may be normal. Therefore, evaluation for a disorder in this pathway should be undertaken in infants with unexplained neurologic disease. Five disorders are considered in this chapter: deficiencies of GTP cyclohydrolase I (GTPCH), recessive as well as dominant forms, 6-pyruvoyltetrahydropterin synthase (PTPS), sepiapterin reductase (SR), dihydropteridine reductase (DHPR), and pterin-4a-carbinolamine dehydratase (PCD) (Figure 16.3). The clinical manifestations of all of them are quite similar, but the carbinolamine hydratase is relatively benign. In addition, variant forms of PTPS and DHPR deficiency exist in which the neurologic signs are either minor or absent. Elevated phenylalanine should initiate investigation in most of these disorders. Sepiapterin reductase, the dominant form of GTPCH I deficiency, and some children with recessively inherited GTPCH deficiency are exceptions in which biopterin is deficient only in the brain [6, 7]. The next step in elucidating a diagnosis is measurement of pterin metabolites in urine or in dry blood spots, and DHPR activity in blood spots. Enzyme activity may be assessed in erythrocytes or cultured fibroblasts. Diagnosis may also be secured by determination of mutations of the relevant gene. Improved prognosis with early therapy makes prompt diagnosis and the timely initiation of therapy important.

In 1974, Bartholome [2] described a patient who was later found to have a block in the synthesis of BH_4. This patient was initially diagnosed as having PKU, but had a progressive deterioration, although dietary restriction of phenylalanine had been exemplary. In the same year, Smith [3] described patients in whom their HPA was atypical in that they had a progressive neurologic illness despite restriction of the intake of phenylalanine. She postulated a disorder in BH_4 metabolism. In 1975, Kaufman and colleagues [4] discovered a problem in cofactor metabolism following demonstration of deficiency of DHPR in such a patient. In 1984, a defect affecting GTPCH 1, the first enzyme in the biosynthetic pathway of BH_4 synthesis, was described [8]. In 1988, a new type of defect affecting BH_4 metabolism was delineated following the detection of a 7-substituted biopterin (as opposed to the normal 6-substituted biopterin due to deficiency of PCD [9]. In 1994, the autosomal dominantly inherited DOPA-responsive dystonia was shown to be associated with dominant mutations in the gene for GTPCH 1 [10]. In 1999, the hitherto last defect in BH_4 metabolism was described that did not lead to HPA. It was first presumed to be a variant of DHPR deficiency [11] but was later shown to be due to SR deficiency [7, 12].

Phenylalanine hydroxylase requires BH_4 for activity in the hydroxylation to tyrosine (see Figure 16.2) [1, 2, 5]. In the conversion of phenylalanine to tyrosine, BH_4 is oxidized to its hydroxyl compound, 4a-carbinolamine. This is recycled to quinonoid dihydropterin in a reaction catalyzed by 4a-carbinolamine dehydratase (PCD) (EC 4.2.1.96).

The oxidized quinonoid dihydropterin compound must be reduced to form BH_4 before it can again be active as a cofactor (Figure 16.2). The reduction is catalyzed by DHPR (EC 1.6.99.7) [4, 5, 13]. The quinonoid oxidation product is unstable and, unless it is promptly reduced,

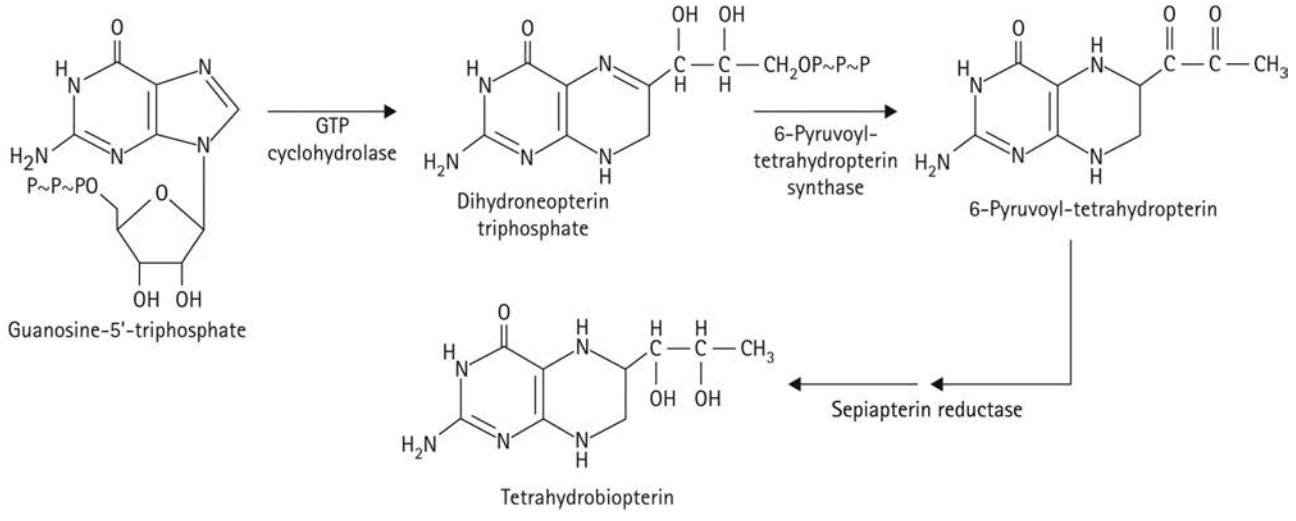

Figure 16.3 Biopterin synthesis; GTP cyclohydrolase I, 6-pyruvoyltetrahydropterin synthase and sepiapterin reductase are sites of clinically defective biopterin synthesis.

it forms a 7, 8-dihydrobiopterin, which is no longer a substrate for DHPR.

The synthesis of the BH_4 cofactor is originally from guanosine triphosphate (GTP), and it proceeds through a number of steps in Mg^{2+}-, Zn^{2+}-, and NADPH-dependent reactions in which reduced neopterin triphosphate (7, 8-dihydroneopterin triphosphate) and 6-pyruvoyl-5, 6, 7, 8-tetrahydropterin are the intermediates (Figure 16.3) [5]. The first committing and rate-limiting step is the GTPCH I reaction (EC 3.5.4.16). The next step is the 6-PTPS (EC 4.6.1.10). SR and the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) are involved in the proper stereospecific conversion of 6-pyruvoyltetrahydropterin to BH_4 and likely also in the pterin salvage pathway by catalyzing the conversion of sepiapterin to 7, 8-dihydrobiopterin that is then transformed to BH_4 by dihydrofolate reductase [14]. Aldose reductases, the 3β-hydroxysteroid dehydrogenase type 2, carbonyl reductases and dihydrofolate reductase may also serve to the conversion of 6-pyruvoyltetrahydropterin to BH_4 [15]. Because of low expression and activity of dihydrofolate reductase and 3β-hydroxysteroid dehydrogenase type 2 in human brain, the biosynthesis from 6-pyruvoyl-tetrahydropterin to BH_4 cannot be completed in the absence of SR, leading to central BH_4 deficiency with normal phenylalanine metabolism in the liver.

All defects have been characterized at the molecular level [16]. The gene for DHPR [17] maps to chromosome 4 (p15.3). The gene for GTPCH is on chromosome 14q22.1-22.2. The cDNA for the human enzyme has been cloned [18]. The cDNA for 6-PTPS has been cloned and mapped to chromosome 11q22.2-23.3 [19]. The gene for PCD has been localized to chromosome 10q22 [19]. Mutations have been identified in the genes for each of the five enzymes defective in human metabolism [16]: DHPR, PCD, GTPCH, PTPS, and SR. These different abnormalities of biopterin metabolism account for less than one percent of patients with HPA in Caucasian populations; however, the incidences are much higher in Turkey, Brazil, China, Saudi Arabia, and Sicily. The disorders are inherited in an autosomal recessive fashion, the exception being the autosomal dominantly inherited form of GTPCH deficiency (DOPA-responsive dystonia).

An international database, BIODEF database, [6, 7] includes over 600 patients; over 350 had 6-PTPS deficiency (56.7%), 217 DHPR deficiency (34.7%), 43 (6.8%) SR deficiency, 31 (4.9%) GTPCH deficiency, and 23 (3.7%) PCD deficiency. Some remain unclassified, indicating the possibility of disorders yet to be identified. Patients with DOPA-responsive dystonia or Segawa disease, which results from autosomal dominant mutations in GTPCH [10] and where HPA does not occur, are only sparsely listed in the BIODEF database.

The spectrum of mutations in these pathways has been summarized by Thony and Blau [16]. Mutations of GCH1 were widely distributed. Only five out of 104 mutant alleles were found in the autosomal recessive form HPA with neurotransmitter deficiencies, while all the other mutants were found in patients with DOPA-responsive dystonia (Segawa disease) expressed as dominant. Mutations in PTPS amounted to 44 alleles scattered widely over the gene. Mutations in PCBD comprised nine mutant alleles, while in QDPR there were 29 mutant alleles. Sixteen different mutant alleles were found in the *SPR* gene [7].

CLINICAL ABNORMALITIES

The clinical manifestations in most of these disorders are indistinguishable except for PCD deficiency. These patients have a much milder phenotype [6, 9]. HPA in the neonatal period has been reported. Minor neurologic signs consisting of slight tremors of upper limbs and hypertonia or hypotonia have been reported in the neonatal period. Only one of 23 patients listed in the BIODEF database needed a substitution with BH_4 and neurotransmitter precursors. They were first recognized on the basis of high urinary neopterin, decreased BH_4 and an unknown compound, which proved to be primapterine, a 7-isomer of biopterin the side chain of which is in the 6 position (see Figure 16.1) [9, 20, 21]. However, several patients were recently found to develop neurologic abnormalities and tendency for developing maturity onset diabetes of the young (MODY) type 3 and insulin resistance in adolescence [22].

The classic presentation of abnormality in BH_4 metabolism is of an infant who appears normal at birth, but is found on screening to have an elevated concentration of phenylalanine in blood. A tendency to low birth weight has been observed in patients with defective synthesis, especially with PTPS deficiency, and newborns frequently have low birth weights and clinical symptoms such as jitteriness but also hypoglycemia [23]. This is less common in reductase-deficient patients. Failure to thrive may be impressive. Development may be normal for two to three months; thereafter, a decrease in activity or a loss of head control may herald the onset of a progressive neurologic degenerative disease. Neurologic signs are progressive with truncal hypotonia, pinpoint pupils, and brisk tendon reflexes as well as movement disorders: hypokinesis, distal chorea, myoclonus, and oculogyric crises (see Figure 17.5). Hypotonia of the trunk and hypertonia of the limbs may develop (Figures 16.4–16.11) [6, 23, 24]. Onset may be with convulsions as early as three months of age [25].

Hypokinesia, marked truncal hypotonia, a mask face, oculogyric crises, myoclonic jerks, and an extrapyramidal tremor develop progressively. The latter three can be mistaken as epileptic phenomena. After infancy, muscle tone increases progressively. Ultimately, these patients develop hypertonia, especially in the lower extremities (Figures 16.11 and 16.12) [25]. There may be bradykinesia, episodic "lead pipe" rigidity, or "cog-wheel" rigidity [24]. The picture may be reminiscent of Parkinson disease. A "stiff baby" syndrome has been described [26] in which

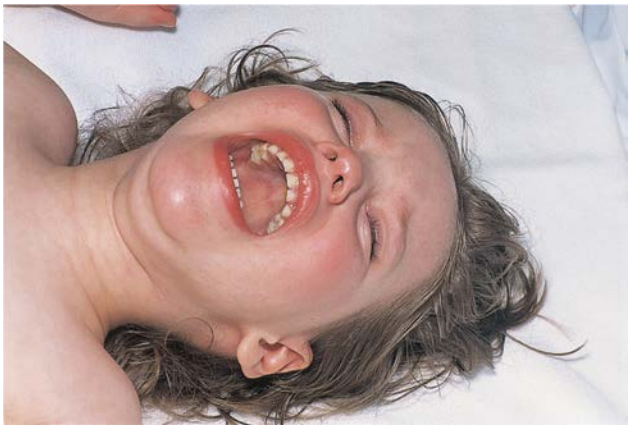

Figure 16.4 GH: A three-year-old girl with defective synthesis of biopterin. The diagnosis was made and biopterin replacement was begun along with 5-hydroxytryptophan, L-DOPA, and carbidopa treatments. Nevertheless, she was significantly neurologically impaired. She could sit unassisted and crawl. Muscle tone was decreased and deep tendon reflexes exaggerated. By six years of age, she had a wide-based ataxic gait and drooled frequently.

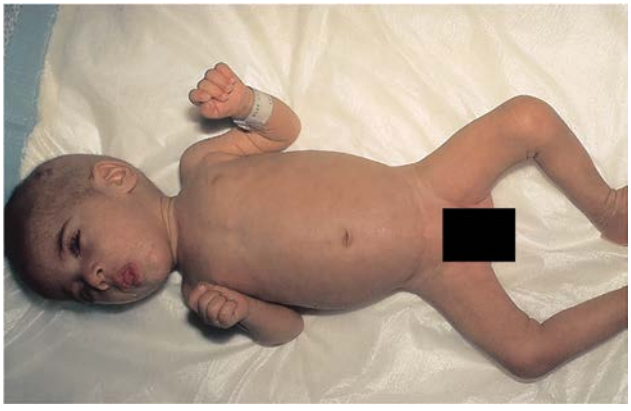

Figure 16.5 RM: A severely affected infant with 6-pyruvoyltetrahydropterin synthase (6-PTPS) deficiency. He had bradykinesia, rigidity, and myoclonus. Color of the skin and eyes were fair. At the age of nine, after treatment with BH_4 and biogenic amine precursors, he attended normal school and had average performance for age and grade.

torticollis was present and progressive rigidity. Episodes of extensor posturing of the extremities and opisthotonic arching of the back are characteristic. The hands are pronated. Deep tendon reflexes are increased. Clonus is frequently elicited and Babinski responses are present [8]. Drooling is a function of difficulty in swallowing and handling secretions. Autonomic symptoms such as hypersalivation, temperature disturbance (in the absence of infection), sweating, drowsiness, and irritability can be very troublesome. Feeding becomes difficult as well. The patient may be unable to swallow even puréed foods [27]. Diurnal fluctuation of symptoms is frequently observed, most likely due to the effects of the concurrent fluctuation of monoamines. Without diagnosis, there may be progressive

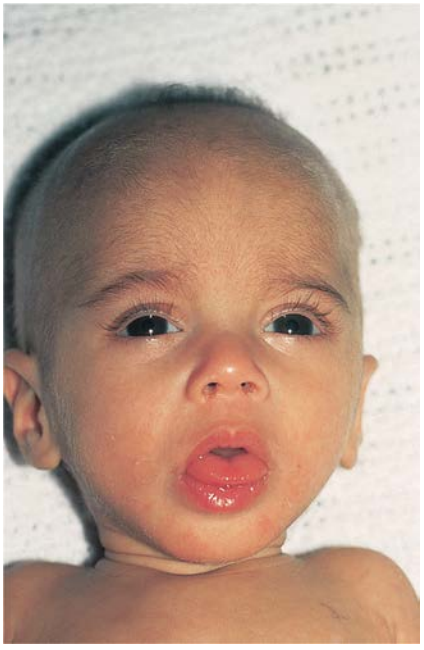

Figure 16.6 Close-up of the face of RM.

Figure 16.7 RM: On the right, 3.5 years later; next to him, his sister who was diagnosed and treated early, was normal neurologically with a normal IQ for age.

neurologic deterioration and microcephaly. The typical patient is withdrawn and appears drowsy or expressionless, but irritability is also characteristic. Involuntary movements may be dystonic in nature. There may be oculogyric spasms which can last for hours (see Figure 17.5). Some patients have tremors. Contractures, failure to thrive, and immobilization may develop; (dystonic) cerebral palsy is a likely descriptive (mis)diagnosis. Seizures are

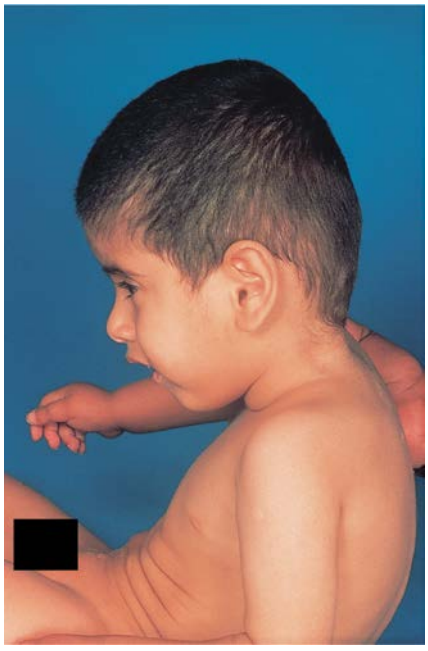

Figure 16.8 AM: Another patient with PTPS deficiency, illustrating the hypotonia. Despite late treatment, he achieved some milestones.

Figure 16.10 IT: An infant with defective BH_4 synthesis detected by newborn screening and treated early was developing well.

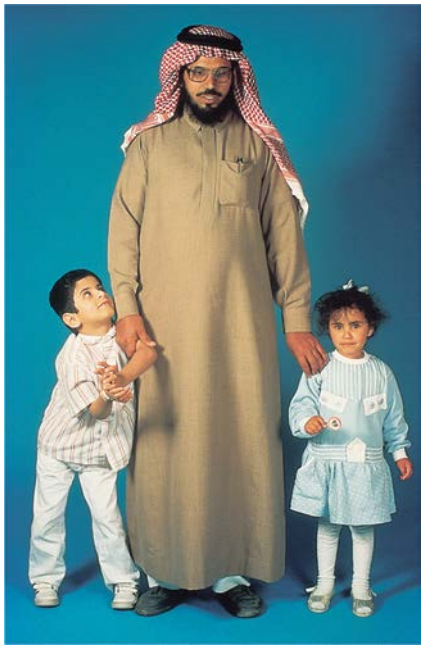

Figure 16.9 AM: Two years later, illustrating his ability to stand with a broad gait and posturing. The sister on the left, also affected, appeared normal, having been diagnosed and treated early.

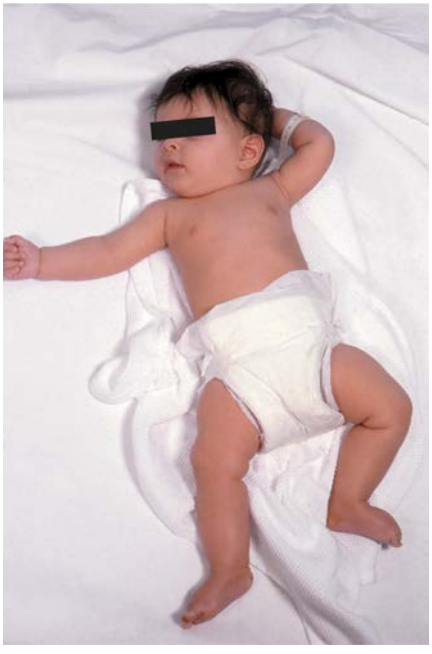

Figure 16.11 A four-month-old Saudi patient with PTPS deficiency who was diagnosed in the newborn period and treated with BH_4, DOPA, and 5-hydroxytryptophan. She has had normal growth and development.

characteristically myoclonic, and myoclonic seizures may be the presenting complaint [24], but grand mal seizures may occur as well. The electroencephalograph (EEG) pattern is abnormal [6]. The impairment in intellectual function is usually profound, but there is heterogeneity, and some patients with only mildly impaired mental development have been described. These have tended to be younger patients, and progressive deterioration of the IQ has been documented, for instance from 83 at 20 months to 24 at 11 years of age [25]. Microcephaly may be a consequence, and computed tomography (CT) scan or magnetic resonance imaging (MRI) of brain may reveal

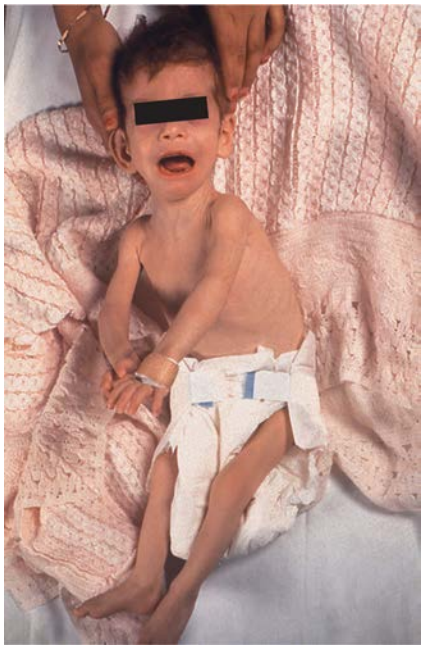

Figure 16.12 The sister of the patient in Figure 16.11 was diagnosed as having spasticity and impaired mental development at four years of age. At six years of age, she died of pneumonia.

cerebral atrophy [28] or lucency of the white matter [29] (Figure 16.13). Abnormalities of the basal ganglia have been described and a pattern of intracranial calcification similar to that of methotrexate toxicity or folate malabsorption [30, 31]. Central folate deficiency can arise in patients with DHPR deficiency and lead to rapid neurologic deterioration despite good control of plasma phenylalanine and treatment of the neurotransmitter deficiency with precursor therapy [32]. This is usually not associated with megaloblastic changes. Brain lesions consist of multifocal, perivascular demyelination in the subcortical white matter accompanied by perivascular microcalcification that is also present in the basal ganglia [32].

Variant forms of PTPS and DHPR deficiency have been described in which the neurologic signs are either absent, minor or late-onset. About 15 percent of patients with PTPS deficiency express a mild phenotype (i.e., initially normal neurotransmitters in the CSF, which is treated with BH_4 monotherapy). Of those, more than 75 percent remain asymptomatic throughout life, but 15 percent develop retardation, dystonia, movement disorders or vegetative symptoms [6]. A late onset case of PTPS deficiency was described where dystonia was the major symptom at the age of 44 years [33]. Two cases with DHPR deficiency have been described in detail [34]. In these, there was some residual activity of the enzyme in fibroblasts. In one untreated child, plasma phenylalanine ranged from 73 to 399 mmol/L, and there were no neurologic signs at 18 months (Figure 16.14). Psychomotor development was normal until 30 months, at which time a deceleration of head growth was observed. In both of these patients, concentrations of homovanillic acid in CSF were always normal, whereas concentrations of 5-hydroxyindoleacetic acid were greatly reduced.

Patients with recessively inherited defective metabolism of BH_4 usually have HPA. Most are detected initially in programs of newborn screening for PKU. However, some children with recessively inherited GTPCH deficiency did not have HPA [6, 35–37]. Severity of clinical presentations were different. Female twins presented with neonatal onset of rigidity, tremor, and dystonia suggestive of a diffuse central nervous system (CNS) involvement; a female presented with typical features of BH_4 deficiency

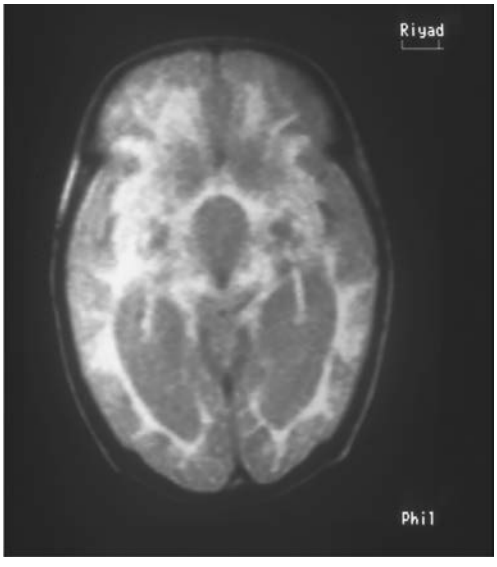

Figure 16.13 MRI of a patient with PTPS deficiency. There was increased T_1 signal intensity in the frontal lobe.

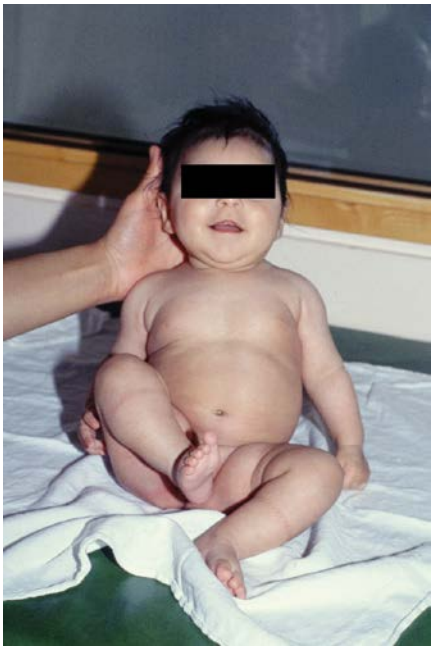

Figure 16.14 10-week old girl with variant DHPR deficiency, diagnosed by HPA, and normal development and neurotransmitter values.

from around 6 months of age, whereas a male presented with a clinical picture more like that seen in the autosomal dominantly inherited GTPCH deficiency. Between the ages of four and six years, he lost motor and speech function, developed generalized dystonia, and symmetrical hyperreflexia with bilateral extensor plantar responses. Oral phenylalanine loading in these patients showed a decreased ability to convert phenylalanine to tyrosine, demonstrating a compromised phenylalanine hydroxylation system in the liver [35–37].

In 1999, two children were described who had the typical clinical course and neurochemical profile of DHPR deficiency. However, HPA was not present, suggesting a form of the disease that is localized to the CNS [11]; these children were later shown to have SR deficiency [12]. Since the original description in 2001, >40 cases of SR deficiency have been described [7]. Symptoms in almost all patients have started in the first few months of life with hypotonia. Subsequent symptoms have included progressive dystonia, chorea, oculogyric crises (see Figure 17.5), tremor, spasticity, microcephaly, growth retardation, depressive and aggressive behavior, and psychomotor retardation. Diurnal variation is usually present. Signs of autonomic dysfunction have included hypersalivation, temperature instability, lethargy, hypersomnolence, and episodes of sweating and pallor. Patients are initially often misdiagnosed as having cerebral palsy, and delay in coming to the correct diagnosis is often many years or decades.

Dominantly inherited GTPCH deficiency, Segawa disease, is also known as DOPA-responsive dystonia. Penetrance is reduced, with the frequency of symptoms being 3-fold to 4-fold higher in females as compared to males [38]. The disease manifests classically with difficulty walking as a result of postural dystonia of one leg, usually within the first decade of life, with the mean age of onset of symptoms being about seven years (range 16 months to 13 years) [39]. Within the next 10–15 years dystonia progresses to all limbs, followed by action dystonia and hand tremor, during which time cognition remains intact. Occasionally, in older children, the first signs may start in the arms or be torticollis or writer's cramp (focal dystonia). The dystonia is frequently asymmetrical. Diurnal fluctuation is normally present, with symptoms improving after nighttime sleep or bed rest. The variation in presenting symptoms is, however, large and may include minor muscle cramps, an early nonprogressive course, delayed attainment of motor milestones, or spastic diplegia [38]. Twenty percent of patients also have hyperreflexia and apparent extensor plantar responses (so-called "striatal toes") mimicking spasticity. There may be parkinsonian features involving reduced facial expression and slowed movements of fingers [40]. Abnormal sleep includes sleeping and nightmares.

DOPA-responsive dystonia, like SR deficiency, does not manifest HPA [41]. A defect in the phenylalanine hydroxylation system can, however, be exposed by administration of an oral phenylalanine load [11, 41, 42]. The disorders are usually diagnosed on the basis of clinical suspicion with an excellent response to levodopa and/or an oral phenylalanine load or low levels of the neurotransmitter homovanillic acid and reduced levels of neopterin and BH_4 in the cerebrospinal fluid (CSF). DOPA-responsive dystonia is eminently treatable with L-DOPA, therefore, its timely recognition is very important.

The outcome in BH_4-deficient patients is highly variable and correlates with the patient's age at start of treatment. Patients not treated, in whom levels of phenylalanine are not kept from being elevated, may develop the fair hair and skin or relative lack of pigmentation that is characteristic of the patient with PKU [24–26]. Some patients have had episodes of hyperthermia without apparent infection [8]. Severe bronchopneumonia may require intensive care [24]. Death occurs often within the first five years of life. One patient died of sudden infant death syndrome (SIDS) in hospital [24]. Overall, mortality documented in the BIODEF database was about 10 percent, most with delayed diagnosis and treatment [6]. When treatment begins in the neonatal period, patients remain frequently asymptomatic or show less retardation, movement disorders and convulsions. Unfortunately, not all patients who are diagnosed and treated early develop normally, and a mild phenotype can shift to a more severe phenotype with age [43].

GENETICS AND PATHOGENESIS

Each of the defects in BH_4 metabolism is autosomal recessive. Consanguinity has been reported [6]. The overall frequency has been estimated at <1 in 500,000 births. In some groups, much higher incidences are seen. In parts of Italy, these defects amount to 10 percent of all patients with HPA, in Turkey 15 percent, and in Southern China and Taiwan 19 percent. In Saudi Arabia, the figure is 68 percent [24].

Levels of DHPR consistent with heterozygosity have been reported [44] in lymphocytes, lymphoblasts, and fibroblasts of parents. Obligate heterozygotes for GTPCH I deficiency were also found to have intermediate levels of activity [45]. In 6-PTPS deficiency, heterozygotes tended to have quite low levels of enzyme activity and may be symptomatic [46]. Prenatal diagnosis has been carried out in DHPR deficiency by enzyme assay [47]. Prenatal diagnosis has most commonly been carried out by the assessment of pterins in amniotic fluid [46, 48]. In GTPCH deficiency, levels of BH_4 and neopterin in amniotic fluid are very low; in 6-PTPS deficiency, BH_4 is low and neopterin high; in DHPR deficiency, BH_4 is high and neopterin normal or slightly elevated [47–49]. Affected and nonaffected fetuses have been diagnosed in this way in DHPR deficiency [48], in 6-PTPS deficiency [47], and in GTPCH deficiency [49]. Molecular diagnosis by restriction fragment length polymorphism (RFLP) analysis of amniocytes has been reported in DHPR deficiency but is now done by DNA analysis where mutations are defined [16].

Five enzymatic defects have been described in this syndrome: DHPR deficiency [4], PCD deficiency [9, 20, 21], and the defects in the synthesis of BH_4, GTPCH deficiency [8, 50], PTPS deficiencies [26, 51] and SR deficiency [7, 12].

Each of these defects leads to a situation in which phenylalanine cannot be converted to tyrosine, even though the phenylalanine hydroxylase apoenzyme is normal. Tetrahydrobiopterin is also the cofactor for the hydroxylation of tryptophan and tyrosine. Thus, its deficiency interferes with the synthesis of serotonin, DOPA, and norepinephrine. Data have been obtained that indicate that this is the case, since levels of 5-hydroxyindoleacetic acid, vanillylmandelic acid, and homovanillic acid in CSF are considerably lower than normal [6, 23]. Low levels of dopamine and serotonin have also been documented in urine [8, 29]. Since it is possible in these disorders to have severe neurologic disease in the presence of only mild HPA, levels of BH_4 may be relatively more sufficient for phenylalanine hydroxylation than that of tryptophan or tyrosine [7, 36, 37]. Defective neurotransmitter metabolism is doubtless related to the genesis of neurologic abnormalities.

6-Pyruvoyltetrahydropterin synthase deficiency

Deficiency of PTPS is the most common of the defects in biopterin metabolism, approximating 60 percent of the patients. The majority have had the typical form, but in ~15 percent, presentation is atypical. The typical patients have high levels of neopterin and low biopterin in the urine (Table 16.1) and CSF. These patients have the highest neopterin levels and the highest ratios of neopterin to BH_4 of all the abnormalities in pterin metabolism. The atypical patients have been referred to as peripheral because the CSF is normal, but with time it can become abnormal [6, 43]. Clinical presentation in these patients is milder and response to treatment more satisfactory. A 20 mg/kg load of BH_4 leads to a rapid decrease in the plasma concentration of phenylalanine.

The enzyme 6-PTPS was formerly called the "phosphate eliminating enzyme", because it catalyzes the elimination of inorganic triphosphate from the dihydroneopterin triphosphate product of the cyclohydrolase reaction (see Figure 16.3). This is an irreversible step. Markedly deficient activity was demonstrated first in biopsied liver [52]. The enzyme is expressed in erythrocytes and typical patients have less than 4 percent of control activity [53]. Patients with the atypical, peripheral form have partial activity in erythrocytes ranging from 5 to 23 percent of normal [54–56]. Residual activity does not always correlate with a milder phenotype; typical patients may have activities as high as 20 percent [46, 48]. Enzyme deficiency can also be documented in fibroblasts [57] in which activity is about one percent of control levels.

The reading frame of the cDNA for 6-PTPS encompasses 435 bp over six exons. Twenty-eight mutations have been found distributed throughout all the exons of the gene [16, 58]. Two were splice-site mutations [57]. There were a few deletions and a majority were point mutations, some producing stop codons. N52S and P87S appear to be common in Asians. Forty-two percent of Chinese patients with PTPS deficiency have a C259T missense mutation, which results in an amino acid change from proline to serine at codon 87. Mild HPA and dystonia were observed in a patient with a homozygous I114V mutation [59].

Table 16.1 Metabolite patterns in enzyme deficiencies

Defective enzyme	Urinary neopterin	Urinary biopterin
GTP cyclohydrolase	Absent	Absent
Pyruvoyltetrahydropterin synthetase	Elevated	Reduced
Dihydropteridine reductase	Normal	Elevated

GTP cyclohydrolase deficiency

Defects in GTPCH account for about 5 percent of those with abnormalities in biopterin metabolism. In these patients, levels of both neopterin and biopterin are low, but their ratio may be normal [6, 8, 45, 55]. Concentrations of neurotransmitters and their metabolites 5-hydroxyindoleacetic acid and homovanillic acid are low in CSF. High concentrations of phenylalanine are corrected with BH_4 loading or replacement.

Defective enzyme activity has been documented in liver, lymphocytes [8, 56], and fibroblasts [60]. The human gene has been cloned and found to span 30 kb in six exons [18]. A mutation converting methionine to isoleucine at position 211 (M211 I) caused deficiency of the enzyme in a patient who was missed on neonatal screening [61]. Missense mutations, such as this and R184H, as well as nonsense mutations (Q110X) lead to complete deficiency of the enzyme. The gene (*GCH1*) is located at 14q22.1-22.2. Some 60 mutations have been reported, which are scattered over the entire coding region in the heterozygous state together with a wild-type allele, and are associated with the dominant DOPA-dependent dystonia [16]. An abnormal gene on one allele may decrease enzyme activity to less than 50 percent, which may produce symptomatic patients and asymptomatic carriers. It is suggested that a difference in the ratio of mutant to wild-type GTPCH mRNA that depends on the locus of a mutation might explain the intrafamilial and interfamilial variation of phenotype seen in dominantly inherited GTPCH deficiency. However, the finding of phenotypic heterogeneity in monozygotic twins demonstrates that other factors are also involved [62].

In DOPA-dependent dystonia patients, the abnormality leads to partial deficiency of BH_4, and subsequently of the activity of tyrosine hydroxylase (TH) [63]. Thus, partial

deficiency leads to decreased dopamine. Age-dependent expression of dopamine receptors may lead to age-dependent clinical manifestations.

Reduced activity of tyrosine hydroxylase in nigrostriatal dopamine neurons may lead to postural dystonia or postural tremor, followed by action dystonia and hand tremor. Parkinson rigidity and resting tremor do not occur. The neuropathology of this disease is striking for an absence of degenerative changes.

Dihydropteridine reductase deficiency

DHPR deficiency accounts for approximately one third of the patients with defects in biopterin metabolism. Levels of neurotransmitters are low in CSF (see Table 16.1). These include homovanillic acid (HVA), vanillylmandelic acid (VMA), 3-methoxyhydroxyphenylglycol (MHPG), and 5-hydroxyindoleacetic acid (HIAA); the metabolites of dopamine, norepinephrine, epinephrine, and serotonin, respectively [64]. Total urinary pterins are elevated and BH_4 is low.

Deficient activity of the enzyme has been documented in the liver, brain, and cultured fibroblasts [4]. The enzyme can also be assayed in erythrocytes [65]. Activity is generally very low, and some patients are cross-reacting material (CRM)-positive and some CRM-negative without correlation with degrees of clinical severity [66, 67].

The gene was mapped to chromosome 4p15.31 [68]. The intron/exon structure has been determined for the gene that codes for a 25.7-kDa protein [69, 70]. More than 50 different mutations have been identified spread throughout *QDPR* (the gene). An insertion of an extra codon for threonine between alanine at position 122 and the threonine at residue 123 was an early identification, but accounts for most of the mutations reported [16, 71]. A number of RFLPs has been identified in the gene, which may be useful for prenatal diagnosis and population genetic studies [69]. Mutational analysis is the best method for prenatal diagnosis in a family with a known mutation [16, 72].

Pterin-4a-carbinolamine dehydratase deficiency

PCD deficiency occurs in about 4 percent of patients with defective BH_4 metabolism [6]. When the phenylalanine hydroxylation reaction takes place, the carbinolamine intermediate is converted to dihydropterin in a reaction catalyzed by the dehydratase PCD. When PCD is defective, there is a conversion to 7-biopterin (primapterin), and the excretion of this compound is a distinguishing characteristic of this disorder [73]. The dehydratase reaction can also proceed nonenzymatically [74] and this could be a reason for the relatively mild phenotype.

PCD is a bifunctional protein with transcriptional function; its gene codes for four exons over 5 kb [75]. Many mutations described have been nonsense mutations, but they have clustered in exon 4 [16].

Sepiapterin reductase deficiency

SR deficiency, the most recently recognized defect, has been documented in 5 percent of patients. SR catalyzes the final step in the biosynthesis of BH_4 by a NADPH-dependent reduction of the two side-chain keto groups of 6-pyruvoyltetrahydropterin (see Figure 16.3). Besides the completion of the *de novo* biosynthesis of BH_4, SR also participates in the pterin salvage pathway by catalyzing the conversion of sepiapterin to 7,8-dihydrobiopterin (7, 8-BH_2) that is then transformed to BH_4 by dihydrofolate reductase [14]. Both reactions consume NADPH. Although SR is sufficient to complete the BH_4 biosynthesis, a family of alternative NADPH-dependent aldo-keto reductases, including carbonyl reductases, aldose reductases, and the 3β-hydroxysteroid dehydrogenase type 2 may participate in the diketo reduction of the carbonyl side chain *in vivo* [15]. Due to low expression and activity of these enzymes in human brain, the biosynthesis from 6-pyruvoyl-tetrahydropterin to BH_4 cannot be completed in the absence of SR, leading to central BH_4 deficiency while phenylalanine metabolism in the liver remains almost normal.

DIAGNOSIS

Although in Caucasians only one percent of the patients found to have HPA have disorders of biopterin metabolism, every patient should be tested for an abnormality of BH_4 because the implications for management and counseling are so different [6, 64].

The diagnosis of these disorders may be made in a number of ways. The quickest is the administration of BH_4. The dose should be 20 mg/kg orally [42]. Administration of BH_4 leads to a prompt decrease to normal in the concentration of phenylalanine in patients with synthesis and reductase defects. It is important that the patient be on a diet containing normal amounts of phenylalanine, not the therapeutic diet employed for patients with PKU. To perform this test, the initial plasma phenylalanine concentration should be above 400 μmol/L (6.7 mg/dL). In neonates with a positive screening result BH4 should be administered approximately 30 minutes before a feed. A few patients with DHPR defects have been missed using the BH_4 loading test [76, 77]. Therefore, loading tests cannot replace metabolite analysis in urine or dried blood spots and must be complemented and confirmed by analysis of enzyme activity. The gold standard for the diagnosis of DHPR deficiency is assay of the enzyme which must be performed in all newborns and children with HPA.

Currently, routine testing for all patients with HPA >120 μmol/L includes urinary pterin analysis and the determination of DHPR enzyme activity in dried blood spots. Alternatively, measurement of pterins can also be performed in dried blood spots on filter paper allowing the analysis of pterins, DHPR activity, and amino acids from one single DBS card [44, 78]. Definitive testing for activity can be accomplished in cultured fibroblasts, lymphoblasts, or freshly isolated lymphocytes. The defect has also been demonstrated in biopsied liver [4].

Pterin metabolites in urine or dried blood spots are measured by high performance liquid chromatography (HPLC) [78–80]. The normal values for biopterin and neopterin in newborns and children are 0.5–3.0 and 1.1–4.0 mmol/mol creatinine, respectively, and the proportion of biopterin is 20–80 percent [81]. In those with defective GTPCH, all pterins are low in blood and urine and the ratio is normal [8, 50]. In patients with 6-PTPS deficiency, the concentrations of biopterin are very low and those of neopterin high. In PCD deficiency, primapterin is formed in the urine, and BH_4 is low. In patients with DHPR deficiency, there is a lack of feedback inhibition, and so there may be massive overproduction of urinary pterins, but the level of BH_4 is always low. On the other hand, patients with DHPR deficiency have been reported in whom urinary pterin analysis was normal [34, 76, 77], indicating further the importance of enzyme analysis in the diagnosis of this condition. The normal plasma BH_4 value is 1.4–3.0; that of blood 2.4–6.0 ng/mL, in CSF 20–60 nmol/L [81].

TREATMENT

The management of the autosomal recessively inherited defects of BH_4 metabolism requires the correction of the serotonin and catecholamine deficiency in the typical forms of the disease and, in addition, prevention of the onset of folate deficiency within the CNS in DHPR deficiency. HPA must also be corrected when present. Blood phenylalanine concentrations should be more rigidly controlled than in classical PKU patients. In BH_4 synthesis defects, BH_4 (5 mg/kg divided in 2–3 doses) is usually sufficient to correct the abnormalities of pterin metabolism (Figure 16.15) in the periphery and to control levels of phenylalanine [6, 32, 34, 81]. This therapy is preferable to the low-phenylalanine diet. Unfortunately, the use of BH_4 is generally not feasible for the treatment of HPA in DHPR deficiency. Large doses (up to 20 mg/kg) are required in the absence of this recycling enzyme [82]. Furthermore, in this disorder BH_4 causes the accumulation of the potentially neurotoxic 7,8-dihydrobiopterin. These patients need a low-phenylalanine diet with close monitoring of phenylalanine levels and supplemental tyrosine (PKU formulations) [76, 81–83].

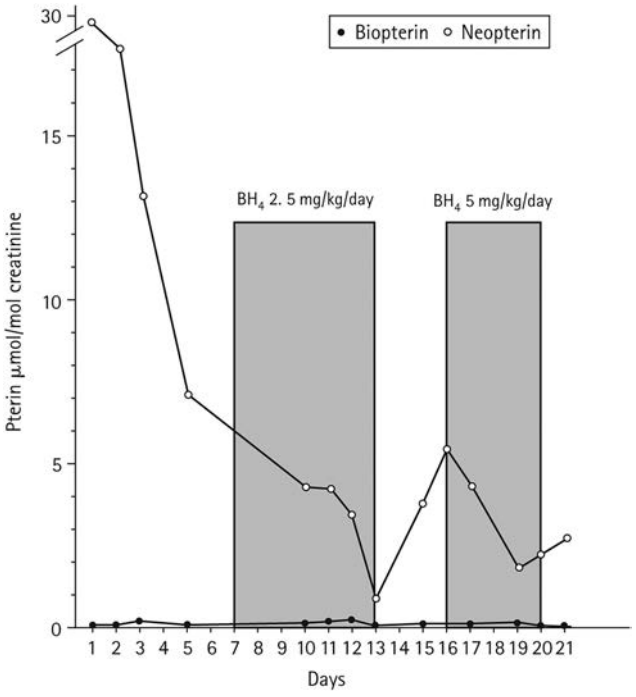

Figure 16.15 Treatment of 6PTS deficiency with BH_4 reduced the excretion of neopterin in the urine.

Sapropterin dihydrochloride (Kuvan®) is the synthetic analog of BH_4. It is the 6R-isomer. It is safe and well tolerated [6, 81]. BH_4 tablets contain 100 mg BH_4. Adverse event rates were similar to those of placebo [84]. It is Food and Drug Administration (FDA) approved for disorders of biotin synthese, as well as for BH_4-responsive PKU.

In addition to controlling the concentration of phenylalanine, treatment of this condition must correct deficiencies of neurotransmitters. This cannot be done with BH_4 alone, although it is clear that it does enter the CSF [6, 81, 85, 86].

Neurotransmitter balance is achieved with a regimen of biogenic amine precursors including 5-hydroxytryptophan and L-DOPA along with carbidopa [6, 64, 81, 87], which inhibits peripheral decarboxylation permitting entry to the CNS, where decarboxylation to serotonin and dopamine takes place. Preparations, such as sinemet, which combine L-DOPA and carbidopa may be useful. In late diagnosed patients, treatment with L-DOPA, carbidopa, and 5-hydoxytryptophan (serotonin) is introduced slowly and sequentially; steps are 1 mg/kg over days or weeks. L-DOPA is given in doses of 8–20 mg/kg and 5-hydroxytryptophan 6–12 mg/kg. Carbidopa is 10–20 percent of DOPA in most formulations. In some patients, a fixed preparation of carbidopa may have to be altered to give more or less carbidopa.

Repeated measurements of neurotransmitter metabolites and pterins in the CSF are required in order to determine optimal doses and to monitor the effectiveness of therapeutic regimens. The level of prolactin in serum is

elevated in these diseases, which is an index of abnormal dopamine homeostasis, and it may be useful to monitor levels in order to guide therapy [83]. Levels of folate may be low in DHPR deficiency, and DHPR appears to have a role in tetrahydrofolate synthesis [88, 89]. Folinic acid has to be employed in doses of 15–20 mg/day in one to two doses [64, 81, 88].

Progressive improvement, with disappearance of myoclonus, involuntary movements, and tetraplegia, has been reported following treatment with biogenic amine precursors [4, 6]. There is evidence that early treatment may prevent progression [6, 24], but overall experience indicates that prognosis should be guarded, especially if not treated as newborns. Many patients have considerable neurologic impairment despite therapy. Programs must be individually tailored to meet the needs of the patient.

Effects of treatment were assessed in a group of Japanese patients with BH_4 deficiency to whom a comprehensive battery of neuropsychological tests were administered [90]. This uncovered deficits in executive function, which has been thought to be controlled by prefrontal dopaminergic systems. Patients treated from birth to 2.5 years had normal executive functioning, while five treated later performed poorly.

In an Italian study of 6-PTPS deficiency [91], patients were divided into those with normal or markedly depressed levels of biogenic amines in the CSF. All patients with the mild form were neurologically normal, except for one with transient dystonia, while all with the severe form had impressive neurologic impairment.

In another study, a dopamine agonist (pramipexole) was used twice daily as an adjuvant of DOPA therapy [92]. The regimen also included selegiline, an irreversible monoamine oxidase (MAO-B) inhibitor, and entacapone, a catechole-O-methyltransferase inhibitor. They reported better results than L-DOPA treatment alone and appreciable reduction in the amounts of DOPA employed.

Treatment of DHPR deficiency is clearly demanding. A good result is by no means guaranteed even if treatment follows all of the guidelines. The Swiss experience indicates that DHPR deficiency is more of a problem than PTPS deficiency. Again, the onset of treatment appears to determine outcome [6, 93].

Maternal PTPS deficiency was documented in a woman who had two pregnancies [94]. In each, doses of neurotransmitter precursors and BH_4 were increased. The first led to a girl who was said to have above average intellectual development despite a left hemiparesis and absence of the corpus callosum and right-sided schizencephaly. The second, a boy, had no anatomic abnormalities and was described as normal at five years of age.

Treatment of DOPA-responsive dystonia with levodopa is enormously rewarding and can lead to a complete and lasting disappearance of the motor abnormalities [39, 63, 64]. If begun early enough, it can also reverse the shortness of stature.

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17

Biogenic amines

Disorders that lead to neurotransmitter imbalance include aromatic L-amino acid decarboxylase (AADC) deficiency, which also leads to both catecholamine and serotonin deficiency, tyrosine hydroxylase (TH) deficiency, and the dopamine transporter deficiency syndrome, which cause catecholamine deficiency. The disorders of BH_4 synthesis (Chapter 16) also lead to abnormalities with the biogenic amine neurotransmitters.

MAJOR PHENOTYPIC EXPRESSION

Progressive developmental impairment, and severe neurologic dysfunction, hypokinesia, truncal muscular hypotonia often combined with limb rigidity, and most noticeably, a progressive extrapyramidal movement disorder, comprising parkinsonism-like dystonia and chorea, oculogyric crises, excessive sweating or temperature instability, potential hypoglycemia, apnea and/or cardiac arrest, along with reduced concentrations in the cerebrospinal fluid (CSF) of 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG). Concentrations of 5-hydroxytryptophan (5-HTP) and 3-O-methyldopa are elevated, and activity of AADC is decreased.

Aromatic L-amino acid decarboxylase deficiency

INTRODUCTION

AADC deficiency was first reported by Hyland and Clayton [1] and Hyland *et al.* [2] in twins who were very hypotonic, and had interspersed bouts of crying and paroxysmal movements of the arms and legs, along with oculogyric crises. They developed abnormalities in temperature regulation and postural hypotension. AADC deficiency was identified as an autosomal recessively inherited disorder of biogenic amine metabolism, which resulted in combined generalized deficiency of serotonin, and all catecholamines. Concentrations of HIAA and HVA in the CSF were very low. Serotonin in whole blood and catecholamines in plasma were also low. Elevated amounts of L-DOPA, 5-hydroxytryptophan (5-HTP) and 3-methoxytyrosine can be present in urine. The activity of AADC (Figure 17.1) was close to zero (one percent of control) in plasma and the liver.

Serotonin and dopamine are formed after the hydroxylation of tryptophan and tyrosine, catalyzed by tryptophan hydroxylase and TH (Figure 17.1) followed by decarboxylation of HTP and L-DOPA by pyridoxalphosphate (PLP)-dependent AADC.

The disorder is relatively rare, but more than 100 patients have been tabulated in a database of pediatric neurotransmitter disorders (www.biopku.org) [3, 4].

An international support group, the AADC Research Trust Children's Charity, has been established as a nonprofit

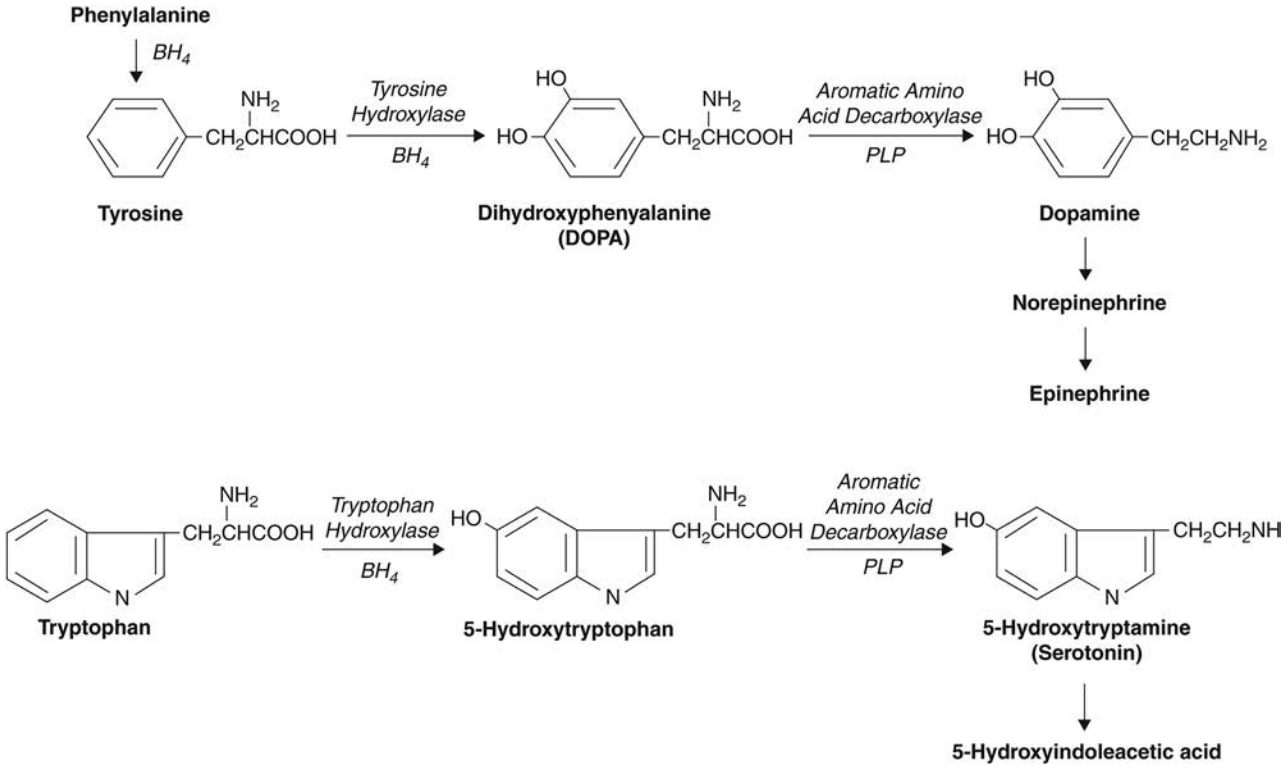

Figure 17.1 Pathways of neurotransmitter synthesis and modifications. The key enzymes illustrated are tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC).

organization that has been instrumental in helping to provide information and to link families and professionals involved in diagnosis, care, and research. The association's board of directors and medical and scientific advisory board review contents, resources, and medical information are posted on the website located at www.aadcresearch.org.

CLINICAL ABNORMALITIES

Onset of clinical manifestations is virtually always (96 percent) [3] in infancy or childhood. In retrospect, most patients had feeding difficulties, lethargy, hypothermia, and hypotension in addition to truncal hypotonia and ptosis as neonates [3, 5]. In 95 percent, axial and truncal hypotonia were present. This may alternate with intermittent limb hypertonia. Head control is poor in infancy. There is extreme irritability, hypokinesia, severe progressive developmental delay, and progressive neurologic dysfunction. Extrapyramidal movement disorder, comprising parkinsonism–dystonia, is obvious from early childhood. Dystonia may be accompanied by athetosis or chorea. Ocular fixation is poor, and 39 percent have had ptosis [3]. Oculogyric crises represent a common (86 percent) and memorable feature of the disease (Figures 17.2–17.5). Diurnal fluctuation, which is a hallmark of Segawa syndrome, is also obvious in many patients with AADC deficiency. A short sleep during daytime can greatly improve symptomatology. Voluntary movements are difficult. Physical examination may reveal brisk knee jerks and extensor plantar responses [6]. Speech is dysarthric and difficult. Before the definitive diagnosis is established, oculogyric crises are often mistaken for seizures, and multiple antiepileptic therapies are initiated, all with little effect on these dopamine deficiency phenomena. Benzodiazepins may ameliorate or stop oculogyric crises, as well as paroxysmal dystonia. After sleep both usually diminish or disappear. In adolescence and adulthood

Figure 17.2 Boy with aromatic amino acid decarboxylase deficiency. He had severe truncal hypotonia and rigidly of limbs, as well as oculogyric crises.

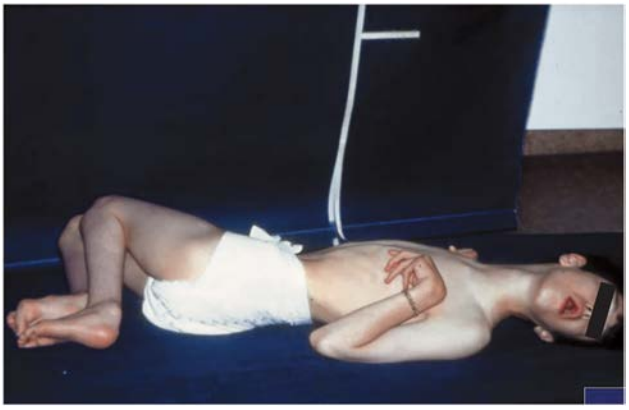

Figure 17.3 An adolescent with a special form of aromatic amino acid decarboxylase deficiency, i.e. L-DOPA responsiveness. He was dystonic and had oculogyric crises.

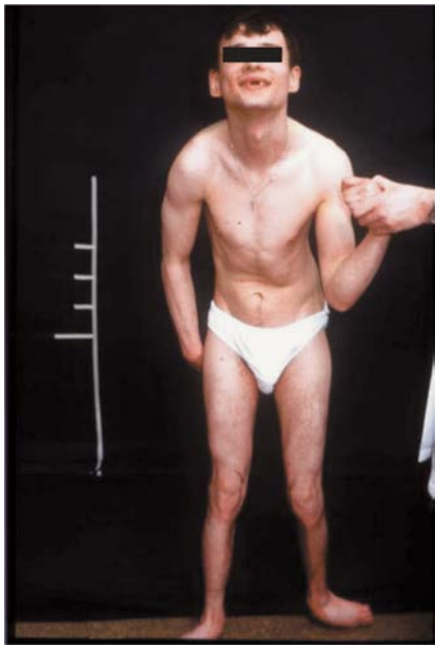

Figure 17.4 The patient shown in Figure 17.3 after administration of L-DOPA.

contractures may develop, and together with slowly worsening osteoporosis require orthopedic attention.

Though the pure neurologic picture is indistinguishable from other dopamine deficiency syndromes, such as tetrahydrobiopterin disorders or TH deficiency, vegetative symptoms such as chronic nasal congestion, hypersalivation and especially complex feeding difficulties related to disturbed intestinal motility, reflux, diarrhea, and/or constipation are often more complex and severe. Patients have difficulty swallowing or eating. This can be so severe that gastrostomy is necessary. Gastroesophageal reflux disease can require fundoplication. Diarrhea and failure to thrive may become the major manifestation [7]. Providing enough energy and fluid is often a problem, and patients

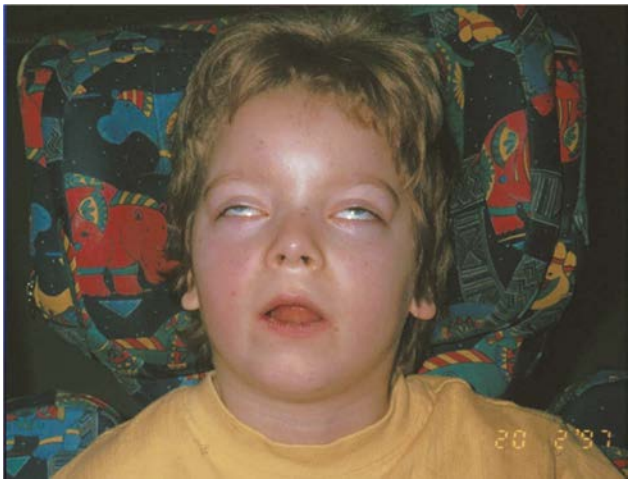

Figure 17.5 A girl with tyrosine hydroxylase deficiency displaying an oculogyric crisis. Reproduced from Hoffmann G.F., Assman B., Bräutigan C., Dionisis-Vici C., Häussler M., de Klerk J., Naunann M., Steenbergen-Spanjers G., Strassburg H.M., and Wevers R.A., (2003), Tyrosine hydroxylase deficiency causes progressive encephalopathy and DOPA-nonresponsive dystonia. Annals of Neurology 54: 556–65, with permission from Wiley.

are at risk of becoming cachectic. Additional autonomic manifestations include excessive sweating and temperature instability. Irritability and insomnia, possibly due to serotonin deficiency, are common. Sleeping disturbances and episodes of excessive crying can be very troublesome and cause demanding problems for families and caregivers. Reduced catecholamine production puts patients at risk for apnea and/or sudden cardiac arrest, especially when the patient is exposed to stressful situations including hospitalization for diagnostic procedures or surgery [8]. Especially in younger children, hypoglycemia can be a problem and in addition orthostatic hypotension when they become older. Insufficient production of growth hormone and response to it provide factors which contribute to hypoglycemia. Patients usually remain significantly below their target height, often below the 3rd percentile. Hyperprolactinemia can lead to amenorrhea and/or lactation in women.

Patients are socially interactive, though impaired mental or motor development, or both, may be major features of the disease (63 percent) [3]. In many cases, cognitive evaluation is not possible because of the severe motor impairment, and cognitive function is always much less impaired than motor functions. Some more mildly affected patients achieve normal limits in cognitive testing.

Individual atypical, milder cases can present later. In one patient hypotonia, short periods of hypertonicity, and oculogyric crises were present but, even without treatment, the child could sit and communicate nonverbally by the age of two years [9]. Three patients (two siblings and a cousin) had syndromic features as well as intellectual disability. They showed craniofacial dysmorphisms, chronic diarrhea with or without recurrent hypoglycemia, gastroesophageal reflux and progressive kyphoscoliosis. They did not display

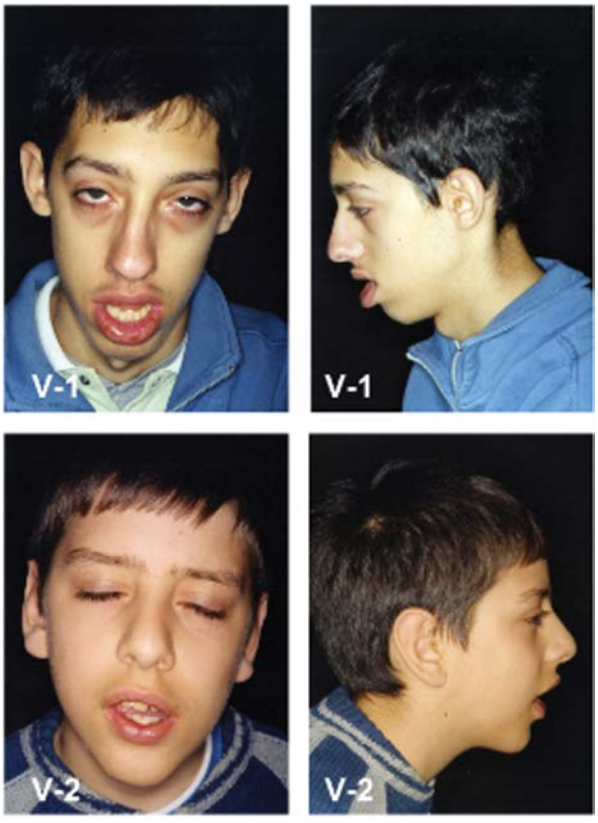

Figure 17.6 Features of two bothers carrying the DDC p.Arg375Cys mutation. Craniofacial dysmorphism include elongated face with long nose, high arched palate, large open mouth with thick lips, prominent chin, malar hypoplasia. (*Illustration kindly provided by Dr. Lidia Garavelli, Reggio Emilia, and Dr. Elena Bonora Bologna, Italy.*)

characteristic clinical features of AADC deficiency, i.e., oculogyric crises or severe hypotonia (Figure 17.6). Only when they became adults was AADC deficiency identified by whole exome sequencing [8]. Another untreated child had autism as the major manifestation [10].

About one third of patients develop single seizures with corresponding abnormalities of the EEG slow or fast waves and polyspikes, but rarely severe epilepsy [3, 11–13]. Seizure types are grand mal or complex focal seizures. It is sometimes difficult to discriminate seizures from oculogyric crises and dystonic spasms.

Imaging of the brain and the EEG have been normal in most patients [3, 12], though delayed myelination in infancy and some generalized atrophy have been reported in individual patients. MR spectroscopy has always been normal.

Psychiatric disorders in carriers

In several series, there was an elevated incidence of psychiatric disorders in relatives of 1st or 2nd degree [3, 11, 12]. These psychiatric disorders were depression, psychosis, suicide or suicide attempts.

The diagnosis of AADC deficiency is dependent on the assessment of neurotransmitters and their metabolites in the CSF. It is necessary to follow a strict protocol for the collection of samples, with special tubes and prompt shipment to the referral laboratory overnight on dry ice [14]. Age-related reference values are critical, as well as recognition of the rostrocaudal pattern of concentrations.

Patterns of neurotransmitter metabolites in the CSF in this and other disorders of neurotransmitter metabolism are shown in Table 17.1. In patients with AADC deficiency, levels of HVA, HIAA, and MHTG are low. Levels of 3-O-methyldopa are markedly elevated. Levels of L-DOPA and 5-HT are elevated, but less so. This pattern was found in 100 percent of patients [3]. The only item in the differential diagnosis of this pattern is a severe reduction of vitamin B_6 observed in deficiency of pyridox(am)ine 5′-phosphate oxidase (PNPO), because this enzyme is responsible for the maintenance of pyridoxal phosphate (PLP) levels in the central nervous system (CNS). Mutations in the *PNPO* gene for this enzyme lead to deficiency of PLP and the same pattern of CSF metabolites as in AADC deficiency [15]. PLP is the cofactor for the AADC enzyme. Measurement of plasma AADC activity or PLP in the CSF will distinguish AADC deficiency from PNPO deficiency. Levels of vanillactic acid and its derivatives vanilpyruvic acid and N-acetylvanilalanine are elevated in urine, and this may be the first clue as to the

Table 17.1 Patterns of metabolites in the CSF in patients with disorders of neurotransmitter metabolism

	Homovanillic acid (HVA)	5-Hydroxy-indoleacetic acid (5-HIAA)	3-Methoxy-4-hydroxyphenylglycol (MHPG)	3-O-Methyldopa
Aromatic L-amino acid decarboxylase (AADC) deficiency	↓	↓	↓	↑
Tyrosine hydroxylase (TH) deficiency	↓	N	N	N
Disorders of BH_4 synthesis (recessive)	↓	↓	↓	N
DOPA recessive dystonia-GTP hydrolase deficiency (dominant)	±↓	±↓	↓	N
Dopamine transporter deficiency syndrome	↑	N	N	N
Pyridoxine phosphate oxidase deficiency (PNPO)	↓	↓	↓	↑

diagnosis as it is found on organic acid analysis of the urine. On the other hand, its elevation may be mild [3, 12]. The compound is formed from transamination of accumulated 3-O-methyldopa. In a pilot study, 3-O-methyldopa is being investigated for newborn screening of AADC deficiency in Taiwan with promising initial results and an estimated incidence of AADC deficiency of 1:32,000 (95% confidence interval: 1:12,443-1:82,279).

Measurement of the urinary concentrations of the neurotransmitters themselves might lead to (paradoxically) normal findings [16]. This may be explained by the enormous capacity of the kidneys to synthesize dopamine, even when only minimal amounts of residual AADC activity are present. Whole blood serotonin is usually diminished. Finally, prolactin secretion by the pituitary is regulated by neurosecretory dopamine neurons in the hypothalamus. Dopamine inhibits secretion, so measurement of an increased serum prolactin may provide a clue to the diagnosis.

Confirmation of the diagnosis of AADC deficiency is by enzyme assay of plasma or cells [3] and finally by mutation analysis. In all patients in whom activity of the AADC enzyme in plasma has been measured, the values were very low or undetectable [3]. The diagnosis can also be confirmed by assay of enzyme activity in biopsied liver [1, 2]. Activities against each substrate were decreased to the same degree.

Preimplantation and prenatal genetic diagnosis has also been accomplished using an amplification refractory mutation system-quantitative polymerase chain reaction technique [17].

GENETICS AND PATHOGENESIS

AADC deficiency is caused by autosomal recessively inherited mutations in the *DDC* gene [3, 18]. Consanguinity has been observed [1, 3, 6]. If the mutation can be detected, this will provide an ideal method for prenatal diagnosis and heterozygote detection.

The human *DDC* gene is encoded by a single-gene that is over 85 kbp in length. It is located on chromosome 7p12.1-p12.3 and contains 15 exons. The enzyme requires PLP as a cofactor and is a homodimer composed of identical subunits with a molecular mass of 53.9 kDa. Differential splicing leads to two forms of *DDC* mRNA that code for a single amino acid sequence. These mRNAs differ in their 5′ untranslated regions and are encoded by two distinct exons, exon N1 being designated the neuronal type and exon L1 the non-neuronal type. The two forms of mRNA are produced by alternative use of these two first exons. Alternative splicing also exists in the coding region of the human *DDC* mRNA.

AADC is required for the synthesis of both serotonin and the catecholamines, and almost all clinical signs and symptoms described above can be attributed to deficiencies of dopamine, norepinephrine, epinephrine, and serotonin. AADC deficiency leads to low levels of all these neurotransmitters and accumulation of 3-O-methyldopa, 5-HTP, and L-DOPA (see Figure 17.1). The methylation of L-DOPA leads to the formation of 3-O-methyldopa. The increased requirement for methyl groups reduces the levels of S-adenosylmethionine within the CNS, and sometimes reduces the levels of 5-methyltetrahydrofolate [19].

Dopamine is synthesized in the substantia nigra, ventral tegmentum, and hypothalamus. Its deficiency affects voluntary movements, cognitive function, and emotion, but also hormonal functions. Dopamine deficiency results in progressive extrapyramidal movement disorders, especially hypokinesia, parkinsonism–dystonia and chorea. Dopamine does not only play a central role in the control of movement, but is also relevant for cognition, emotion/affect, and neuroendocrine, pituitary gland hormones (prolactin and growth hormone). Under physiologic circumstances, dopamine concentrations are also high in the kidney, where it is involved in control of sodium and water transport. In the gastrointestinal (GI) tract, dopamine controls motility. Reduction or inappropriately low response of norepinephrine and epinephrine can lead to hypoglycemia, ptosis, and autonomic disturbances with loss of regulation of body temperature, vascular tone and blood flow, decreased blood pressure, and inappropriate response to stress. It also affects attention, mood, sleep, and cognition. Deficiency of serotonin appears not to lead to severe neurologic symptoms, but affects appetite, sleep, memory, learning, mood, and modulation of pain mechanisms, as well as cardiovascular and endocrine functions. This description outlines the pathophysiology for the very complex, variable, severe and difficult to treat consequences of AADC deficiency.

Homozygosity for a mutation in the AADC was found in the original twins of Hyland and Clayton [5]. A total of 24 mutations have been found in 49 patients [3], and about 50 are tabulated in the BIOMDB database (www.biopku.org) [4]. The most common mutation, found in 45 percent of alleles, was IVS6+4A>T, followed by p.Ser250Phe with about 10 percent [3]. Patients with an IVS6+4A>T mutation so far were all of Chinese origin, where more patients appear to have been diagnosed and usually suffer from a severe phenotype. Otherwise genotype–phenotype correlations have been elusive. Additionally, discovered missense mutations include p.L38P, p.Y79C, p.A110Q, p.G123R, and p.R412W [3]. Two frameshift mutations were p.I42fs and p.I433fs.

TREATMENT

The therapeutic management is very challenging. No systematic studies on drug therapy are available, and there are no clear guidelines or therapeutic recommendations. Striking improvement in tone and mobility was reported [1, 2] in the twins initially reported following treatment with a monoamine oxidase inhibitor, a dopamine antagonist, and pyridoxine. Hyperdopaminuria found

in these patients increases with treatment with L-DOPA [16]. Medication with documented success in at least some patients are compiled in Table 17.2. All of them have to be prescribed, "off-label" and without established pediatric dosage regimens. Programs of treatment (Table 17.2) usually include dopamine agonists, pyridoxine, and monoamine oxidase inhibitors [3, 5, 12, 20]. A suggestion of sex differences was found [5] in a series of five male patients who responded well to treatment and progressed developmentally while five females and two males responded poorly. Outcome does appear to be better in males than in females [3]. About half of the patients stabilize on individual treatment regimens and achieve different degrees of motor and psychosocial skills. About 20 percent improve to the point that they successfully manage regular schooling as long as some help is provided for the remaining motor handicap. Others do not show any sustained improvements [3, 5, 12, 13]. Despite therapeutic interventions, the disease course is often severe and may be fatal at a young age [3, 21].

Specific therapy is aimed at correcting monoaminergic and, in parts, catecholaminergic neurotransmission, utilizing the following pharmacologic strategies: dopamine receptor agonists, nonselective or selective monoaminoxidase (MAO) inhibitors, alpha-adrenergic agonists (in general as nose drops), catechol-*O*-methyl transferase (COMT) inhibitors, therapeutic doses of the cofactor of AADC (pyridoxine or PLP) and melatonin [20].

First-choice medications appear to be dopamine agonists, such as bromocriptine or pramipexole, or ropinirole, followed by MAO inhibitors such as selegeline or tranylcypromine. An additional trial with the anticholinergic drug trihexyphenidyl can be helpful. Pergolide and cabergoline should not be used because of the high risk of fibrotic complications. Amantadine with 4 mg/kg/d can help against drug induced dyskinesias.

Pyridoxine has been used in doses from 150 to 4800 mg/day (20–160 mg/kg/d) divided in two or three doses, and recently an increase was observed of the expression level of the enzyme by pyridoxine in patients with the common mutation, S250F [22]. Treatment with the immediate precursors L-DOPA and 5-HTP has generally been ineffective [3]. A trial with B_6 and L-DOPA monotherapy, but not the usual combination of L-DOPA with a decarboxylase inhibitor such as carbidopa, might be considered in all patients with AADC deficiency. The response should be monitored clinically and by CSF analysis of neurotransmitters. Nevertheless, three siblings responded dramatically to L-DOPA [12]. Figures 17.3 and 17.4 illustrate such a response. Sequencing revealed a homozygous G-to-A substitution converting glycine to serine at position 102 (G102S) in exon 3 in this family. Kinetic studies and analysis of the protein structure revealed that the mutation increased the apparent K_m for L-DOPA, altering the protein configuration near to the substrate-binding site [12, 18]. An excellent response to a monoamine oxidase inhibitor and a dopamine agonist was reported in two siblings with an "unusually mild phenotype" [23].

Folinic acid is recommended in doses of 10–20 mg/day, because of the possibility of cerebral folate depletion resulting from methylation of accumulated L-DOPA.

Nasal congestion is a frequent problem for AADC patients probably because of their deficit in catecholamines. Topical application of oxymetazoline or xylometazoline is necessary in most cases. The package insert, as well as major textbooks, warns that the use of the combination of MAO inhibitors and topical alpha-adrenoreceptor agonists nose drops may cause severe hypertensive crises. In AADC patients, however, catecholamines are reduced and in practice we are not aware of any such complication using this combination in our patients.

Gene therapy has been reported as a novel therapeutic approach for AADC deficiency [21]. With the aid of targeted neurosurgical procedures, viral vector-mediated gene transfer of the human AADC gene into the putamen has become possible. The first results of this exciting technique seem promising, and further trials are underway.

Table 17.2 Treatments in AADC and TH deficiency

Agent	Mechanism	Additional aspects including side effects partially specific to these patients
Bromocriptine	Dopamine agonist (D2>D1)+ other receptors	Fibrosis of heart, lung, retroperitoneum
Pramipexole ropinirole, rotigotine	D2 > D1 agonists	Rotigotine to be applied via transdermal patches
Tranylcypromine	Nonselective MAO-inhibitor	Sleep disturbances, especially if administered late in the day, "cheese syndrome"
Selegeline	Selective MAO-B-inhibitor	
Pyridoxine	Can optimize residual AADC activity	Increases gastric acidity
Trihexyphenidyl	Anticholinergic	Anticholinergic effects

Source: Adapted from Hoffmann GF, Assmann BE. In: Hoffmann GF, Blau N (eds). *Congenital Neurotransmitter Disorders.* New York: Nova Science Publishers; 2014, 65–80, where details of dosages and applications are given.

Tyrosine hydroxylase deficiency

MAJOR PHENOTYPIC EXPRESSION

Deficiency of tyrosine hydroxylase (TH) results in isolated dopamine/catecholamine deficiency. Milder manifestations of DOPA-responsive dystonia, hypokinetic rigidity, oculogyric crises have been assigned as the cause of autosomal recessive Segawa syndrome, type A, but many patients present with a more severe and complex early onset progressive hypokinetic-rigid syndrome plus dystonia and mental impairment, type B. Low CSF HVA and MHPG; deficiency of TH and mutations in the TH gene. Autosomal dominant Segawa syndrome is caused by mutations in the guanosinetriphosphate cyclohydrolase (*GCH1*) gene (Chapter 16).

INTRODUCTION

In 1995, Ludecke and colleagues [24] reported a patient with what they called recessively inherited L-DOPA responsive parkinsonism in infancy. Onset was at three months with involuntary jerky movements followed by generalized rigidity and few spontaneous movements. By six months, the face was expressionless and the tongue tremulous. Drooling was prominent, and there was bilateral ptosis. Cogwheel rigidity was noted. The dopamine metabolite, HVA, was reduced to very low concentrations in the CSF, and genetic deficiency of TH was confirmed by demonstrating a homozygous point mutation in exon 5 (L205P). Ptosis was improved by the ocular instillation of phenylephrine. Treatment with L-DOPA and carbidopa led to increase to normal CSF, HVA and dramatic improvement in hypokinesis and the other parkinsonian features.

Marked improvement in response to treatment with L-DOPA was also reported in four Dutch patients with impaired motor development, hypokinesis, truncal hypotonia, masked facies, and rigidity of limbs due to a different point mutation in exon 6 (R233H) [25, 26], as well as seven different novel mutations in children with the initial diagnosis of spastic paraplegia [27].

A different, more severe presentation was reported [28] in an Italian infant with onset at birth of progressive hypotonia, hypokinesia, dysphagia, extensive sweating, and irritability. He had dysphagia and reduced facial expression. Movements were dystonic. Concentration of HVA in the CSF was undetectable. Response to L-DOPA was limited.

In a study of 36 patients and review of the literature [29], distinction was made of the more common infantile onset DOPA-responsive phenotype as type A, which has become incorporated into classifications of dystonias as the cause of recessive L-DOPA-responsive dystonia (DYT5), and the neonatal onset DOPA-nonresponsive type B leading to progressive infantile encephalopathy. The authors recognized that there was likely a spectrum of phenotypes between the two types. They pointed out a patient reported as an example of type A who also had many of the features of type B [30]. Virtually all patients had onset by one year of age, the few exceptions by five years [29] which is very different from the autosomal dominant Segawa syndrome, which typically presents in childhood with walking problems due to dystonia of the lower limbs. Diurnal fluctuation, which is a hallmark of autosomal dominant Segawa syndrome, is not as prominent in TH deficiency. Mutations were almost exclusively missense [29]. This led to the conclusion that more severe disruptions of the gene may not be compatible with life.

Tyrosine hydroxylase catalyzes the rate-limiting step in the formation of the catecholamines, dopamine, norepinephrine, and epinephrine (see Figure 17.1). Deficient activity of the enzyme leads to diagnostic decrease in CSF concentrations of the metabolic catecholamine degradation products, HVA and MPHG (see Table 17.1). The gene TH contains 14 exons and has an open reading frame of 1491 bp [31]. It was mapped to chromosome 11p.15.5, the most distant end of 11p [32].

CLINICAL ABNORMALITIES

Deficiency of TH leads to clinical manifestations very early in life, nearly all by the first-year birthday [24, 25, 28, 29]. The clinical features are such that the diagnosis is mostly not made clinically, but usually by the pattern of metabolites in the CSF (see Table 17.1), so lumbar puncture is an essential step in elucidating the diagnosis. Most patients present with a phenotype that is characterized by a progressive extrapyramidal movement disorder (hypokinetic-rigid syndrome with dystonia) (TH deficiency type A, the recessive DOPA-responsive dystonia). Some can develop normally until an arrest of motor development with a combination of neurologic symptoms before or around one year of age. Hypokinesia, marked truncal hypotonia, mask face, oculogyric crises (see Figure 17.5), myoclonic jerks, and an extrapyramidal tremor develop progressively. The latter three can be mistaken as epileptic phenomena. After infancy, muscle tone increases progressively. Contractures, failure to thrive, and immobilization may develop; (dystonic) cerebral palsy is a likely descriptive (mis-)diagnosis. Some patients who did not develop extrapyramidal symptoms in the first year of life were able to walk independently and followed a clinical course best summarized as spastic paraplegia [27].

Their symptoms resolved completely following L-DOPA supplementation, and they continued to live as healthy and independent adults. Others may have no pyramidal tract or ocular signs but progressive extrapyramidal symptoms mainly dystonia and rigidity [33]. In several patients, TH deficiency has led to infantile-onset parkinsonism [33].

At the severe end of the clinical spectrum, there are virtually no movements, including no dystonic movements (TH deficiency type B) [28, 29, 34]. The first clinical impression of these infants, who generally hold a frog-like position, is that of a neuromuscular disorder (see Figure 17.7a). However, increased deep tendon reflexes and pyramidal tract signs point to cerebral dysfunction. Miosis may be present but may go undiagnosed because of prominent ptosis (see Figure 17.7b). We have followed and treated one patient for more than 20 years. Initially, he developed devastating pick-dose dyskinesias to L-DOPA in anything but homeopathic doses. Over many months, smallest increases of L-DOPA were possible which after many years could finally be increased up to 8 mg/kg/day. The patient achieved unsupported walking when 11 years old. In contrast with the relative improvement of motor functions, mental and language development remained severely impaired and, at the age of 17, he was a hyperactive boy with impulsive, iterative behavior, severe intellectual impairment and absence of communicative skills. Similarly, the girl depicted in Figure 17.5 survived more than a decade before she was mobile in a wheelchair and achieved some education. Tragically, her brother had suffered from the same neurometabolic disease, but at that time, a definitive diagnosis could not be reached. A therapeutic trial of L-DOPA had been attempted on him, but discontinued because of severe dyskinesias, and he had died at age nine. In severe TH deficiency, extremely gradual rising of dosage together with patient long-term clinical observation can allow major improvements and even a satisfactory outcome but can take years.

In the GI tract, dopamine controls motility. Absence of norepinephrine can lead to hypoglycemia, ptosis, and autonomic disturbances among which is decreased blood pressure, and such patients also show symptoms of intestinal dysmotility, diarrhea, hypoglycemia and inadequate stress responses. There is an obvious tendency to preterm birth with troublesome cardiorespiratory perinatal adaptation. It is reasonable to assume that some patients die undiagnosed perinatally or even prenatally. After the neonatal period, difficult-to-control and potentially life-threatening paroxysmal periods of general malaise with lethargy and vegetative symptoms of irritability, sweating, and drooling can occur. Growth can be compromised and bone age severely delayed, suggestive of an impaired secretion pattern and/or stimulation of growth hormone.

Neuroimaging was normal in the majority of patients, in 20 of 29 studied [29]. Nonspecific mild changes in white matter signaling were found in 19 percent of type A and 63 percent of type B patients. Gross abnormalities of structure or signal intensity were never seen in the MRI.

The pattern of catecholamines in the CSF is distinctive (see Table 17.1). Absence of reduction of HIAA distinguishes it from AADC deficiency. The HIV:HIAA ratio is useful, and it correlates well with the degree of clinical severity.

Prolactin concentration may be elevated in the serum, as in AADC deficiency. In one patient, galactorrhea was described [35]; this result of hyperprolactinemia, also a consequence of dopamine deficiency, was present before the development of neurologic features of the disease.

GENETICS AND PATHOGENESIS

The disease results from mutations in the TH gene and is transmitted in an autosomal recessive fashion. The iron-containing mixed-function oxidase requires molecular oxygen and BH_4 for activity. The enzyme is expressed in the central and peripheral nervous systems, in particular, in the brain and the adrenal medulla but also in a few non-neuronal tissues, such as the kidney, intestine, and lymphoid nodes [29]. Documentation of deficiency of the enzyme is therefore difficult and most documentation has been via mutational analysis. In the original family with the Q412K mutation, the enzyme was demonstrated to have lowered affinity for tyrosine and residual activity of about 15 percent of control [36].

Some 37 different mutations have been observed [29]. Of 100 alleles, 96 were affected by missense mutations leading to amino acid substitutions. Pathogenic mutations have been identified in the promoter region of the TH gene [37]. The most common mutations were c.698G>A in the

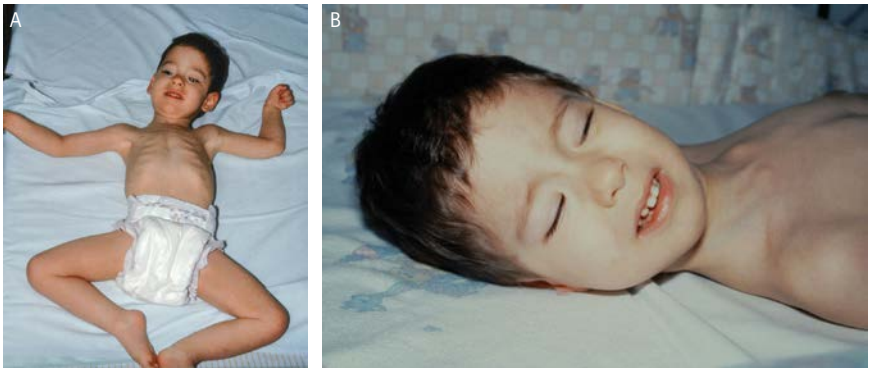

Figure 17.7 Three-year old patient with type B TH deficiency with severe hypokinesia, hypotonia, dystonic posture of upper limbs, (A) reduced facial mimicry and (B) ptosis.

Dutch population [25, 26] and c.707T>C [29] in the Greek population. Both appear to have resulted from founder effects. Genotype–phenotype correlations were not evident in these two, and all others have been private mutations.

Only four patients have had stop codons leading to truncation of the protein, and no patient has had homozygosity or compound heterozygosity for two of these. The patients with the promoter mutations had type A phenotypes.

TREATMENT

Treatment of the central deficiency of dopamine with L-DOPA has been the treatment of choice, and virtually always with carbidopa [25, 29, 34]. The most readily available preparations contain carbidopa or benserazide. Therapeutically successful doses of DOPA employed have been 3–10 mg/kg per day, but it is advisable to start with 0.1–0.5–1 mg/kg divided into three or four doses, and to increase weekly. Hypersensitivity to DOPA has been especially observed in type B patients; some tolerated only 0.5 mg/kg and some none. Inhibitors of dopamine degradation, such as selegiline, have been employed (see Table 17.2). Patients who tolerate a reasonable dose of L-DOPA generally displayed a good or moderately good response. Improvement in movement, hypokinesis, tremor, rigidity, and dystonia are often dramatic, permitting impressive improvement up to completely normal motor function in some. Children who had been wheelchair-bound for years have walked [29]. Clinical improvement is lasting, and patients have been followed over decades. Most patients have not had CSF levels of HVA monitored, but improvement clinically was found, even despite a failure to return CSF HVA to normal.

Tardive dyskinesia is a recognized side effect of treatment, usually responsive to a reduction in dosage.

Moderate mental impairment was judged to occur in follow up in 33 percent of type A patients and 91 percent of type B [29].

Dopamine transporter deficiency syndrome

MAJOR PHENOTYPIC EXPRESSION

Progressive severe infantile parkinsonism-dystonia, axial hypotonia, pyramidal tract signs, oculogyric crises and a complex eye movement disorder consisting of ocular flutter together with saccade initiation failure. Milder onset with progressive juvenile parkinsonism. Extremely elevated dopamine metabolites in CSF and loss-of-function mutations in SLC6A3.

INTRODUCTION

Infantile parkinsonism-dystonia is typically caused by inborn errors of metabolism affecting the dopamine biosynthetic pathway including pterin defects (see above and Chapter 16), and thus represents disorders of dopamine deficiency. In 2004, Assmann and colleagues described two girls and one boy with a novel combination of clinical and biochemical features [38]. In these patients, severe infantile parkinsonism-dystonia was associated with extremely elevated dopamine metabolites in CSF and a complex eye movement disorder of ocular flutter together with saccade initiation failure. Pyramidal tract signs emerged in the course of the disease. In 2009, Kurian and colleagues identified the underlying cause in autosomal recessively inherited loss-of-function mutations in SLC6A3, encoding the dopamine transporter, the principle regulator of the amplitude and duration of dopamine neurotransmission [39]. Combining these results, as well as additional patients, the term dopamine transporter deficiency syndrome was coined [40]. The disease added "transportopathy" to the well-established concept of "channelopathy" as a fundamental mechanism of neurologic disease. Later, a small number of milder, later onset cases were identified, presenting in childhood/adolescence with progressive (juvenile) parkinsonism [41, 42]. To date, approximately 25 patients with DTDS have been identified [40, 41, 42]. Treatment is very challenging and until now, no pharmacologic agents seem to stop disease progression.

CLINICAL ABNORMALITIES

In retrospect, nonspecific features of irritability and feeding difficulties are reported from the neonatal period in infants with dopamine transporter deficiency syndrome [38, 40]. Axial hypotonia and abnormal movements become obvious from 1–7 months of age. In the first months of life, some patients exhibit chorea/dyskinesia, some dystonia, some hypokinetic parkinsonian features and/or variable combinations. Over time, parkinsonian features steadily increase, and predominate together with bradykinesia/akinesia, severe generalized dystonia, a coarse, predominantly resting, distal tremor and hypomimia in older children (Figure 17.8). In the majority of patients, there are orolingual dyskinesia as well as characteristic abnormal

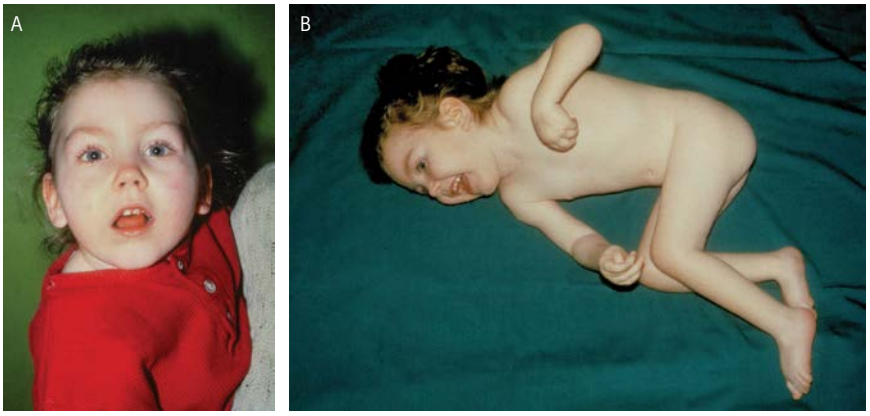

Figure 17.8 Four-year old girl with dopamine transporter deficiency syndrome. From birth, she was irritable and difficult to feed. By four months, clear abnormalities of limb tone and abnormal horizontal eye movements were noted. By 10 months, she had developed an asymmetrical dystonia and chorea. By four years (photos) she had rigidly flexed limbs, was clearly bradykinetic, which modified the chorea, and now had orobulbar involvement. Note drooling and the posture of all limbs caused by increased tone. She died at age 16.

eye movements: saccade initiation failure (inability to make rapid, abrupt eye movements) in addition to ocular flutter (involuntary bursts of horizontal bidirectional saccades of the eyes around a fixation point). Oculogyric crises and eye lid myoclonus were less common. The disease is progressive with severe generalized dystonia, rigid limbs, marked bradykinesia, and recurrent dystonic crises. The cognitive development is relatively spared and despite anarthria, children are able to understand everyday conversation, acquire general concepts, recognize some written words and develop methods of nonverbal communication. With time, all patients developed bulbar dysfunction and required nasogastric/percutaneous endoscopic gastrostomy feeding. Gastroesophageal reflux may require Nissen's fundoplication. Orthopedic complications such as limb contractures, kyphoscoliosis and hip dislocation are frequent, particularly later in the disease course. In the most comprehensive series published to date, more than 60 percent of patients had been mistakenly diagnosed with dyskinetic spastic and/or mixed cerebral palsy. As CSF neurotransmitter analysis is not regularly performed in such children it appears likely that many still remain undiagnosed.

The hallmark of the dopamine transporter deficiency syndrome are persistently highly elevated concentrations of homovanillic acid (HVA, the dopamine metabolite) in CSF and elevated dopamine, while 5-hydroxyindoleacetic acid (5-HIAA, the serotonin metabolite) is normal, resulting in a characteristic elevation of the ratio of HVA:5HIAA. In all patients investigated, the ratio varied between 5 and 15 (normal <4). In repeat investigations of the same child, we have observed a slight decrease of the ratio with time but no correlations between the HVA:5-HIAA ratio and the phenotype. Serum or urine HVA are usually slightly elevated or at the upper end of reference while serum prolactin is mostly normal.

A milder, later presenting phenotype following a normal early neurodevelopmental course was reported from two families with juvenile or adult onset progressive parkinsonism-dystonia [41]. The mildest known patients are adults who, after a normal childhood, developed attention deficit hyperactivity disorder and subsequently manifested with tremor and progressive parkinsonism-dystonia in adulthood [42]. Analysis of neurotransmitters was normal.

The prognosis of dopamine transporter deficiency syndrome is guarded. All of the first patients published by us in 2004 have succumbed at 9, 15 or 16 years of age following secondary complications such as pneumonia, aspiration and unexplained death during sleep, though one patient with early onset disease has been reported at 34 years of age [41]. All of the milder affected patients survived.

GENETICS AND PATHOGENESIS

Definitive diagnosis of the dopamine transporter deficiency syndrome is by demonstrating homozygous/compound heterozygous pathogenic variants of the *SLC6A3* gene located at 5p15.33 [43]. Dopamine is synthesized in the pre-synaptic dopaminergic neuron, packaged into synaptic vesicles and transported to the synaptic membrane for release into the synapse and action at dopaminergic receptors at the post-synaptic membrane. The dopamine transporter actively retrieves dopamine from the synaptic cleft and is the principal regulator of dopaminergic neurotransmission.

The mature, glycosylated dopamine transporter has a molecular mass of 85 kD, whereas immature nonglycosylated DAT has a molecular mass of 55 kD and does not transport dopamine as efficiently as the mature protein [44]. The dopamine transporter consists of 12 transmembrane protein domains (functioning as a gated channel with two conformations, open either exclusively to the extracellular or intracellular milieu [45]. Binding of the extracellular substrate dopamine to its primary binding site results in a conformational change from outward-facing to inward-facing relocating dopamine into the presynaptic neuron. Medications that inhibit the dopamine transporter include methylphenidate, amphetamine, pemoline, as well as cocaine. Several studies investigated possible variants of the dopamine transporter with attention-deficit hyperactivity disorder, bipolar disorder and heritable influences on cigarette smoking. The results were only partially conclusive. A *de novo* DAT

missense mutation, Thr356Met, was identified in a patient with autism [46].

In the dopamine transporter deficiency syndrome, dopamine is not taken up from the synapsis into the presynaptic neuron as documented in one patient after administration of the dopamine transporter ligand ioflupane and missing transport activity visualized with single photon emission CT DaTSCAN [40]. Failure of dopamine re-uptake decreases dopamine available for repackaging into and release from synaptic vesicles in the presynaptic neuron. In addition, dopamine is left in the synaptic cleft with downstream signaling effects on dopaminergic receptors and D2 autoreceptors further inhibiting dopamine synthesis.

A variety of mutations have been reported in patients with the dopamine transporter deficiency syndrome, including deletions, missense, nonsense, and splice-site variants. The first pathologic variant identified was a homozygous 1184C-T transition in exon 9 of the *SLC6A3* gene, resulting in a pro395-to-leu (P395L) substitution in a highly conserved residue of the E4B loop close to transmembrane domain-8 [39]. *In vitro* functional expression assays in HEK293 cells showed that the P395L-mutant protein had no dopamine reuptake activity. There are, as yet, no common variants identified. There is no clear genotype–phenotype correlation in the patients with infantile, but the late-onset patients harbor mutations with residual dopamine transporter activity. The Asp421Asn mutant identified in ADHD and adult onset parkinsonism specifically disrupts a sodium binding side in DAT causing anomalous dopamine efflux instead of uptake [42].

TREATMENT

Patients with genetic defects in the dopamine biosynthesis pathway usually respond at least in part to dopaminergic treatment strategies. In contrast, patients with the dopamine transporter deficiency syndrome appear to be resistant to therapeutic attempts, and pharmacotherapy shows limited partial benefits at best. Some patients benefitted from dopamine agonists with high affinity to the presynaptic autoreceptors, such as Pramipexole and Ropinirole [38, 40].

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18

Homocystinuria

MAJOR PHENOTYPIC EXPRESSION

Ectopia lentis, vascular occlusive disease, intellectual disability, osteoporosis, accumulation of homocystine and methionine, and defective activity of cystathionine synthase.

INTRODUCTION

Homocystinuria was first described in 1962 by Carson, Neill and colleagues [1, 2]. The enzymatic defect was identified by Mudd and colleagues [3], two years later. Since then, considerable experience has been developed which has defined the clinical phenotype, the abnormal biochemistry, and the natural history of the disease [4].

The molecular defect is in the enzyme cystathionine synthase (EC 4.2.1.22) (Figure 18.1). This enzyme is on the metabolic pathway for methionine, and patients may be recognized by an increase in the concentration of methionine in the blood. This property forms the basis for the inclusion of homocystinuria in programs of routine newborn screening. In some patients, accumulation of methionine may give a prominent, unpleasant odor. The clinical picture regularly includes many features, like subluxation of the lenses of the eyes, which are characteristic of a disorder of connective tissue. Extreme variability of clinical presentation is a consequence of whether or not there are thrombotic events, and if so, which areas of the body suffer infarction. Variability also results from the fact that there are two distinct populations of homocystinuric patients, one of which responds to treatment with pyridoxine and one that does not [4].

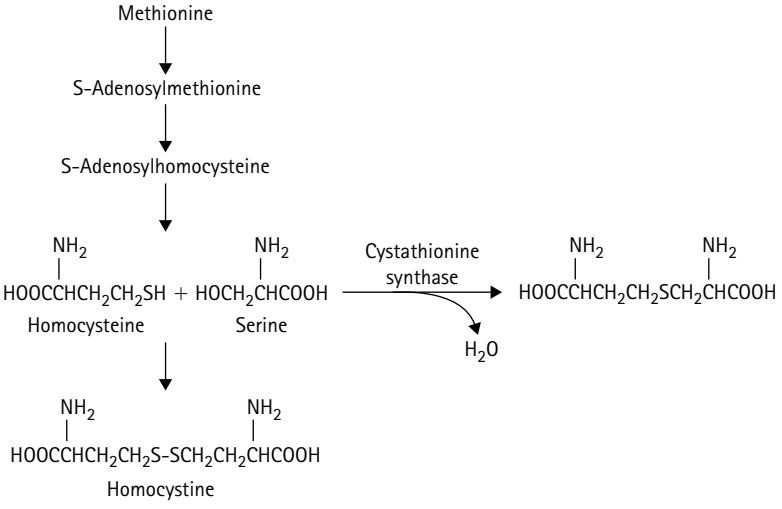

Figure 18.1 In homocystinuria, the defective enzyme is cystathionine synthase.

CLINICAL ABNORMALITIES

Homocystinuria represents a multisystem disorder with involvement of the eyes, integument, skeleton, vascular system, and nervous system [4, 6, 7]. Patients are born normal but in the untreated, severe form they develop the full constellation of clinical abnormalities within 2–6 years of life. Ectopia lentis is a striking and readily recognizable manifestation of the disease (Figures 18.2–18.5). It is often preceded by rapidly worsening myopia, which may be the only manifestation [4–8] and, by 38 years of age, only 3 percent of patients have both lenses in place. Dislocation is usually present by ten years of age. The dislocation is said to be usually, but not always, downward – the opposite of the situation in Marfan disease. Its presence may be signaled by iridodonesis, a dancing or shimmering of the iris. Electron microscopy reveals partially broken zonules, abnormal zonular attachment, and a spongy capsular

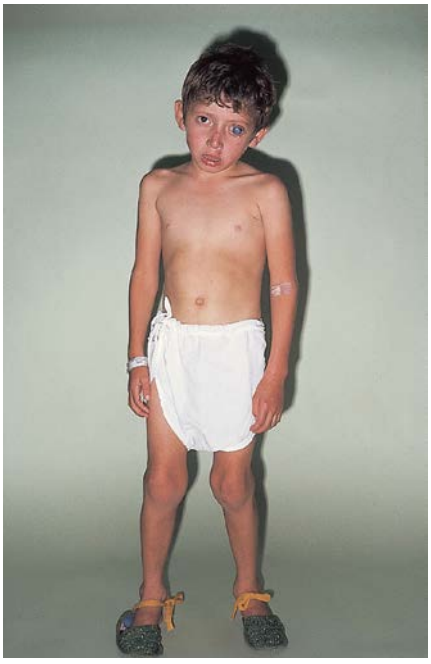

Figure 18.2 MG: A six-year-old boy with homocystinuria. He had short stature and genu valgum.

Figure 18.3 Closer view illustrates MG's eyes. Subluxed lenses had previously been removed bilaterally, after which he developed glaucoma in the left eye. He had fair skin and hair and a pronounced malar flush.

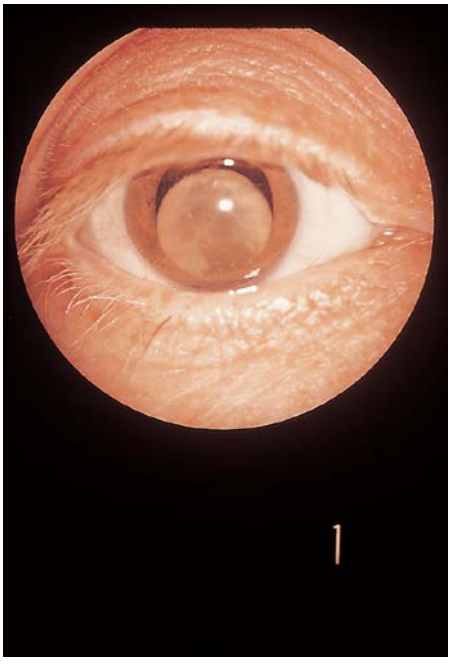

Figure 18.4 The dislocated lens in homocystinuria is usually downward, while in Marfan syndrome it is upward.

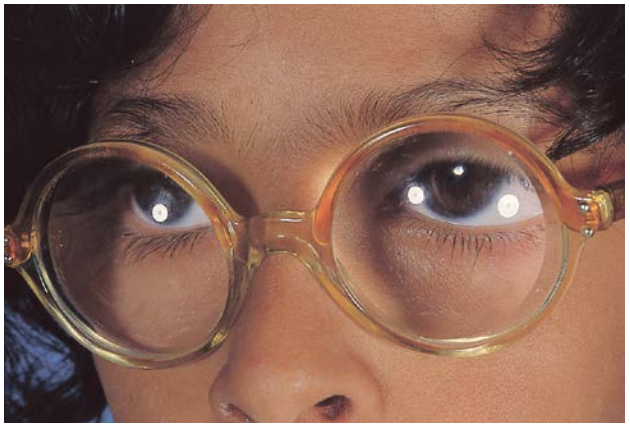

Figure 18.5 NMM: A ten-year-old girl with homocystinuria. She had been found one year previously to have left-sided glaucoma and subluxed lenses bilaterally. The left lens was removed.

appearance [8]. Complications may include dislocation into the anterior chamber and papillary block glaucoma (Figure 18.3). Other ocular abnormalities include myopia, optic atrophy, cataracts, or retinal detachment [9].

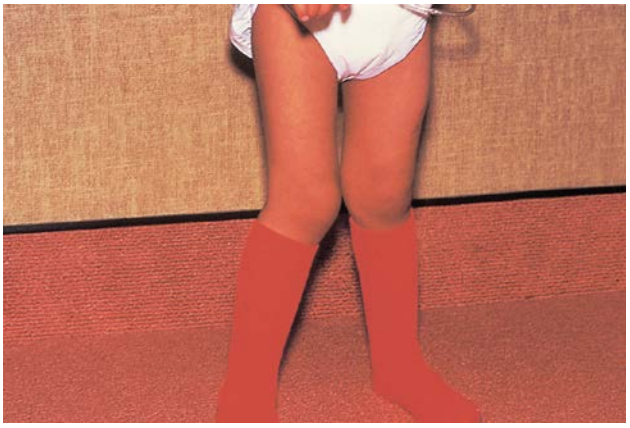

Figure 18.6 Pronounced genu valgum in a three-year-old with homocystinuria.

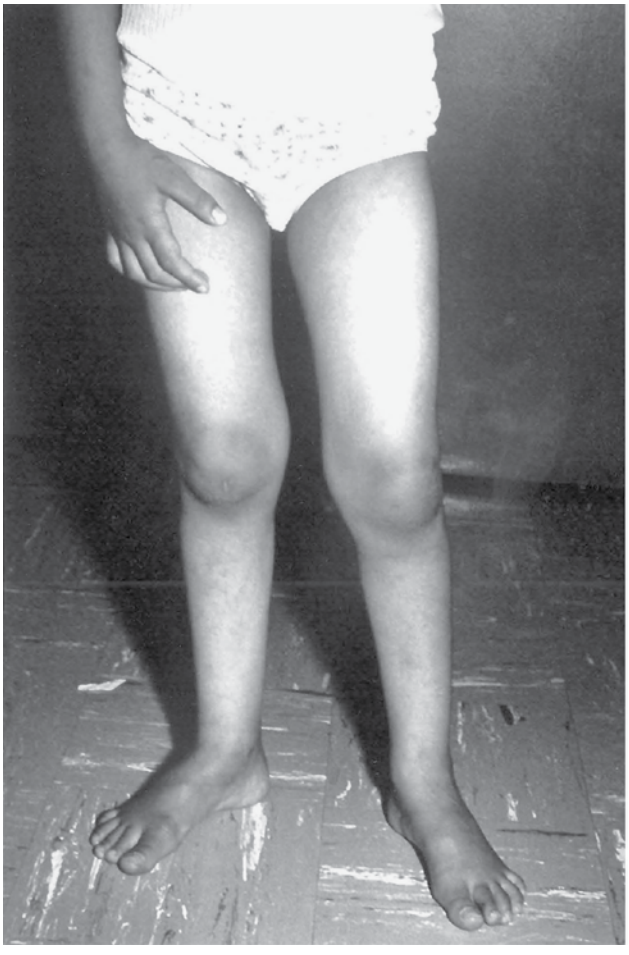

Figure 18.7 Genu valgum in a patient with homocystinuria. The ankles were also in valgus and the feet everted.

The pigmentation of the iris may be lighter than in other family members, and the same may be true for the skin and hair. A pronounced malar flush [10] was first recognized in Ireland, but we have also seen it in patients with considerable cutaneous pigment (Figures 18.2 and 18.3). The skin may otherwise have blotchy erythema and pallor, and livido reticularis [11] is particularly common in the distal extremities, which may be quite cold and show other evidence of vascular instability.

Skeletal abnormalities are prominent, especially genu valgum (Figure 18.6). Valgus may also be present in the ankles, often along with pes cavus. The feet may be everted (Figure 18.7). Some patients may be tall and thin and have a marfanoid appearance (Figures 18.8 and 18.9), but true arachnodactyly is rare, and some patients have a failure to thrive or shortness of stature. Pectus excavatum or carinatum may be present (Figure 18.9). There is a generalized osteoporosis. This is the most common musculoskeletal change, and 50 percent of

Figure 18.8 NMM: She appeared tall and thin and height was in the fifth percentile. She had long, thin fingers.

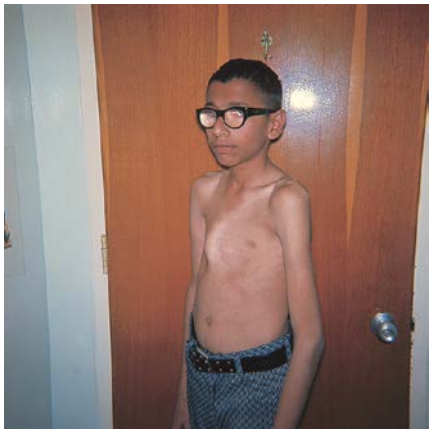

Figure 18.9 A marfanoid appearance in a patient with homocystinuria. He had a prominent pectus carinatum and very thick corrective lenses.

patients have osteoporosis by the end of the second decade. Roentgenograms (Figures 18.10–18.12) characteristically reveal platyspondyly. There may be posterior biconcave or fish-mouth appearance, and there may be impressive compression fractures or kyphoscoliosis [12, 13].

Impaired mental development is common but not invariable. About 50 percent of untreated patients have intellectual disability [4, 14]. Presenting as developmental delay during the first years of life, intellectual disability is often the first recognized sign of homocystinuria. Focal neurologic disease is due to cerebrovascular accidents. Thrombotic or vascular disease appears as a central pathomechanism of disease involving the nervous system. In patients responding to pyridoxine, the mean IQ was 78, while that of nonresponders was 64 [4]. IQ scores among affected siblings were similar [14]. Seizures occurring in about 20 percent of patients and abnormalities of the electroencephalogram (EEG), also common, probably also reflect the variable nature of vascular accident in this disease. Many patients have been observed to have typical strokes, with transient or permanent hemiplegia. A number of patients have had polymyoclonus, dystonia and parkinsonism, not caused by basal ganglia infarction [14–19]. Spasmodic torticollis may usher in ultimately fatal dystonia.

Psychiatric abnormalities have been observed in more than half of one series of 63 patients [14]. Three children were reported to have folate-responsive periodic behavior, including rage attacks [20, 21]. A three-year-old had episodic repetitive behavior thought to represent psychomotor seizures [20]. Adults have been diagnosed as schizophrenic or depressed [14], or to have personality disorders.

Neuroimaging by computed tomography (CT) or magnetic resonance imaging (MRI) may be normal until the occurrence of cerebrovascular disease. Evidence of infarction has been obtained in patients presenting with hemiparesis, with or without papilledema. Cerebral venous and dural sinus thrombosis has been demonstrated by CT scan and confirmed by digital subtraction angiography [22].

We, and others, have observed diffuse leukoencephalopathy as a rare finding in classical homocystinuria. Neurologic symptoms were not always present. However, in the presence of severe hypermethioninemia greater than 1000 μmol/L, leukoencephalopathy could be reversed by lowering methionine. Brain edema rather than demyelination was the most likely cause [23, 24]. Neuropathologic study has revealed occlusion of vessels, old and new thrombi, spongy degeneration, and neuronal loss [25, 26]. In the patient who died at 18 years of age with dystonia, the brain was histologically normal [16].

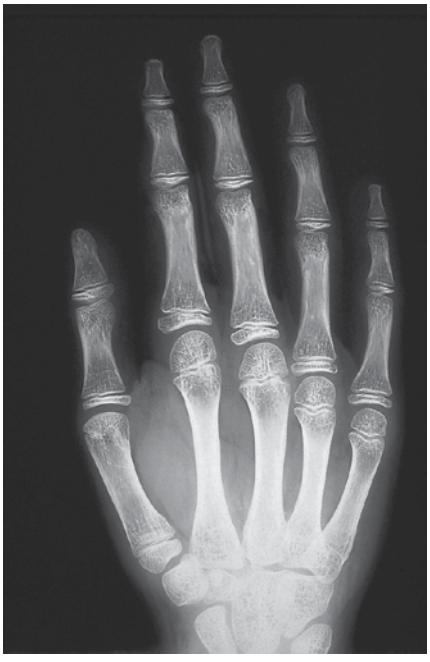

Figure 18.10 Roentgenogram of the hand of the patient shown in Figures 18.5 and 18.8 illustrates the arachnodactyly.

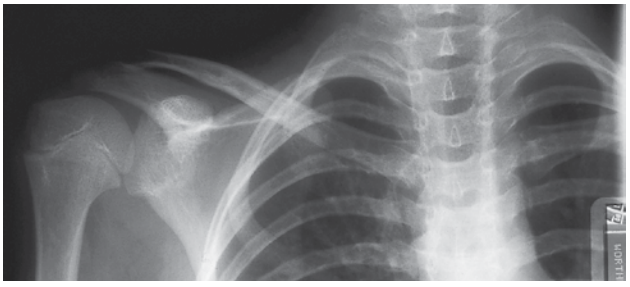

Figure 18.11 Roentgenogram of the spine of the same patient revealed osteopenia and compression of thoracic vertebrae.

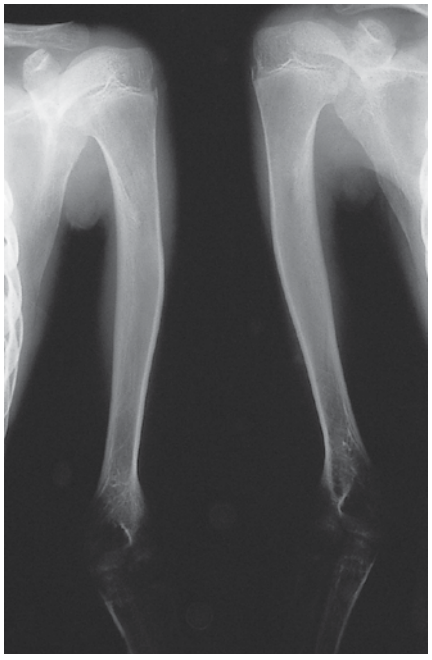

Figure 18.12 Roentgenogram of the humeri of a 12-year-old girl with homocystinuria revealed osteopenia and lateral bowing.

Vascular disease in homocystinuria can occur in any vessel and at any age, including infancy, involving the vessels themselves, as well as a tendency of the blood to clot (Figure 18.13). Thromboses may be arterial or venous, and may be fatal. Cerebrovascular disease occurred in one third of 147 patients, and 32 percent of those thromboembolic events were strokes [4]. Ten patients had myocardial infarction. There were 11 percent peripheral arterial occlusions, 51 percent peripheral venous occlusions, and 32 of these patients had pulmonary emboli. Surgery may be especially strongly associated with thromboembolic accidents [4, 17, 25]. Medial degeneration of the vessels and intimal proliferation of both narrow vessel lumens, and initial injury is followed by the adherence of unusually sticky platelets. The end result is severe narrowing of the arteries. This may be demonstrated angiographically, as may aneurysmal dilatation [27].

Acute pancreatitis has been reported [28] in two patients, one of whom developed a large pseudocyst that was drained surgically. Spontaneous pneumothorax has been reported in three patients with this disease, one of whom had two episodes [29, 30].

Homocystinuria and pregnancy have been reported [31] in 11 women (15 pregnancies), six of them pyridoxine responsive and five nonresponsive. Complications of pregnancy included pre-eclampsia in two and a venous thrombosis in a leg in one. There were two spontaneous and one induced abortion. Of 12 live-born infants, ten were normal and two had multiple anomalies, one of which was the Beckwith-Wiedemann syndrome, which were judged to be unrelated to homocystinuria. Experience has grown to more than 100 pregnancies especially with the pyridoxine-responsive form of the disorder showing that there is no significant risk of malformations in the offspring. Nevertheless, pregnancies in CBS-deficient women should be carefully monitored and awareness of increased risk of maternal thromboembolism. The risk is particularly high for six weeks after delivery, while the uterus is involuting.

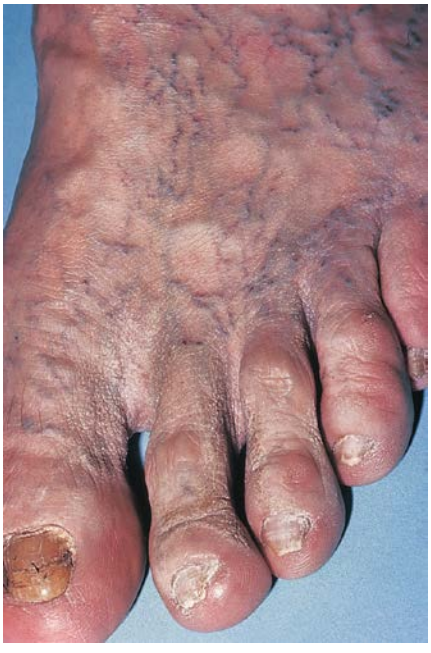

Figure 18.13 Prominent venous pattern in the feet of a man with homocystinuria.

GENETICS AND PATHOGENESIS

Deficiency of cystathionine synthase is autosomal recessively transmitted. It occurs with a frequency of one in 1800 newborns in the state of Qatar [32], one in 50,000 in Ireland and New England and one in one million in Japan; overall frequency is between one in 200,000 and 350,000. The defective enzyme may be demonstrated in cultured lymphocytes and fibroblasts, as well as in tissues such as the liver. Ranges of activity are from zero to ten percent of the control mean. Pyridoxine-responsive patients always have some residual activity, and increased activity of hepatic enzyme has been documented in response to treatment. Three types of enzyme were delineated: zero residual activity; reduced activity and normal affinity for pyridoxalphosphate; and reduced activity and affinity [33]. In a study of fibroblasts of 20 patients, each of 14 with residual enzyme activity had cross-reacting material (CRM) [34]; in six with undetectable activity, three had no CRM and three had reduced amounts of CRM.

The enzyme cystathionine β-synthase is a tetramer of 63 kDa [35], which undergoes post-translational proteolytic increase in activity with decrease in size to 48 kDa. It has binding sites for pyridoxal phosphate, as well as homocysteine and serine. S-adenosylmethionine and heme are activators [36, 37]. In most patients, enzyme size is normal, but exceptions have been encountered [38].

The locus for human cystathionine β-synthase was mapped to chromosome 21 in Chinese hamster-human cell hybrids [39], and cDNA prepared from immunopurified mRNA was used to verify the locus at the subtelomeric region of chromosome 21q22.3 [40], where it is syntenic with α−A-crystallin. There are 23 exons over some 28 kb [41], from which the 551 amino acids are encoded by exons 1–14 and 16. Alternate splicing may include exon 15, which is represented in a few mRNA molecules, but its 14 encoded amino acids are not found in the expressed enzyme. There is also alternative splicing among five exons (designated −1a to −1e) in the 5′-untranslated region. More than 170 mutations have been identified [42], and the functional consequences in many have been confirmed by expression systems. Among the first to be identified was a G to A change at 919 in exon 8, which converts glycine 307 to serine [43], and this mutation is the leading cause of homocystinuria in Ireland. This, and the pyridoxine-responsive I278T, were the most common of 310 homocystinuric alleles [44].

The mutation c.1006C>T (p.R336C) in exon 11 is a founder mutation responsible for the high frequency of homocystinuria in the State of Qatar, 1:1800 [32]. The

mutation involves a cytosine to thymine transition at a CpG dinucleotide known to be hypermutable and has recurred independently in different populations. Clinical observations, enzyme measurements and *in vitro* expression studies showed that this mutation completely abolishes enzyme activity and is not associated with vitamin B_6-responsiveness. Another point mutation in exon 8 is a T to C transition at position 833, causing a substitution of threonine for isoleucine 278, which is the predominant mutation in the Netherlands and in Italy, and is associated with a pyridoxine-responsive phenotype when it is homozygous, and may or may not be when present in a compound heterozygote. Another frequent alteration, is a splice mutation in intron 11, 1224-2 A>C (IVS 11-2 A>C), which results in the skipping of all of exon 12. Interestingly, about half of the point mutations in the coding region originate from deamination of methylcytosine in CpG dinucleotides [44], and nearly one quarter of the point mutations are found in exon 3, the most highly conserved region of the gene.

Most patients have been compounds of two different mutant alleles, and most mutations are private [44]. Among compounds, a pyridoxine-responsive patient had the I278T mutation, as well as a 135-bp deletion that deleted 45 amino acids from 408 to 453 [45]. This patient had been previously found to have one abnormally small polypeptide subunit [38]. An interesting mutation [46] in a pyridoxine-responsive patient homozygous for G1330A changed aspartate 444 to aspargine and abolished the regulatory stimulation of activity by S-adenosylmethionine. A lack of correlation between genotype and phenotype is exemplified by three siblings with the same molecular defect, one of whom had a single episode of claudication in the calf as his only clinical manifestation, while the other two had marked defects in intellectual function and skeletal changes [47].

A novel type of mutation was reported [48] in patients with homocystinuria and premature thrombosis, but without ectopia lentis or any of the other abnormalities of connective tissue. These were p.I435T, p.422L, and p.S466L, located in the noncatalytic terminal region of the synthase gene, coding for enzymes that were catalytically active, but lacking in response to S-adenosylmethionine. These observations raise the possibility that the structural abnormalities of this disease may not be caused by elevated levels of homocysteine.

Among 11 families in Georgia, USA, p.I278T and p.T353M amounted to 45 percent of mutated alleles [49]. The former was exclusively Caucasian and B_6-responsive, while the latter was exclusively Black-American, and unresponsive to B_6. In Denmark, most of those homozygous for p.I278T were either unaffected clinically or had only a thromboembolic event after the third decade [50]. None had skeletal or other connective tissue abnormalities.

Deficiency of the enzyme leads to the accumulation of homocystine and its excretion in large amounts in the urine. Patients generally have elevated concentrations of methionine in blood, and newborn screening programs have been based upon blood methionine levels [51]. However, 20 percent to 50 percent of pyridoxine non-responsive cases and even many more of pyridoxine non-responsive cases are missed by this approach [32, 52]. Total homocyst[e]ine is a much more sensitive analyte, but requires a longer analytical procedure and higher costs. Met to Phe ratio as first-tier and Hcy as second-tier appears to be the optimal strategy [53]. Screening for urinary homocystine in the past was most readily carried out by using the nitroprusside tests (Figure 18.14) [54], which tests for the excretion of sulfur-containing amino acids, or by staining a paper chromatogram or electropherogram with iodoplatinate. The diagnosis can be confirmed by quantification of the amino acids of the urine, where homocystine and the mixed disulfide of cysteine and homocystine are found. Since the major portion of homocysteine in plasma is bound to protein [55], the preferred method is to determine total plasma or serum homocyst[e]ine by adding a reducing agent to release-bound homocysteine prior to deproteinization, after which high performance liquid chromatography (HPLC) with detection of a fluorescent thiol reagent [56] or mass spectrometry [57] may be used. The sample should be centrifuged within one hour if stored at room temperature since red blood cells generate homocysteine. After centrifugation total homocyst[e]ine is stable for at least four days at room temperature, for several weeks at 4°C and several years at –20°C.

Heterozygosity has been documented by the assay of cystathionine synthase in lymphocytes, fibroblasts, and liver [3]. Prenatal diagnosis has been accomplished by assay of the enzyme in cultured amniocytes, and affected fetuses have been detected [58]. Activity in normal chorionic villus material is so low that prenatal diagnosis by this method is precluded.

Heterozygote detection has been carried out by enzyme assay of cultured fibroblasts, but there is overlap between

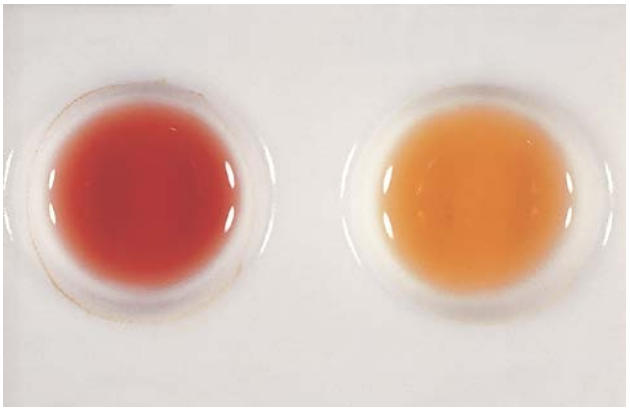

Figure 18.14 Positive cyanide nitroprusside test on the left indicating the excretion of larger than normal amounts of sulfhydryl-containing amino acid. The normal negative test is shown on the right.

carrier and controls [59]. As many as 90 percent of carriers have been estimated to be detectable by measuring peak plasma levels of homocystine after an overload of methionine [60].

TREATMENT

Lifelong treatment can undoubtedly ameliorate the disease and optimal long-term outcome depends on its earliest possible introduction [4, 61, 62]. Pyridoxine responsiveness should be determined in all patients with homocystinuria, and those who respond should be treated with a target of total homocyst[e]ine <50 μmol/L. This may not be achievable for patients that only show a partial and especially no response. This is the major feature currently determining prognosis [4]. Of six patients treated from the neonatal period, IQ scores ranged from 82 to 110 [4]. Evidence that early treatment inducing good control of levels of homocysteine (<11 μmol/L) prevents mental impairment was reported [61] in experience with 23 patients, 19 diagnosed by newborn screening. Of 13 compliant patients, mean IQ was 106, which matched that of ten controls, while those poorly compliant had a mean IQ of 81; two untreated patients had IQs of 52 and 53. Similarly, in a genetically homogenous group of patients with B_6-unresponsive homocystinuria and neonatal diagnosis treatment, avoided any visual problems and resulted in normal IQ, while problems in receptive and expressive language were observed [62]. It is also clear that reduction of levels of homocystine with pyridoxine will prevent thromboembolic events [4]; thromboembolic complications are decreased among those who respond to pyridoxine even in those treated late. Doses have ranged from 100 to 1200 mg/day and should be determined individually. Peripheral neuropathy has occurred in individuals treated with large doses of pyridoxine [63]. Doses up to 500 mg/day appear to be safe. A recommendation was for 300–600 mg/day [64]. Patients requiring larger doses to reduce levels of homocystine should certainly be monitored with tests of nerve conduction. Folate deficiency should be avoided by concomitant treatment with folate. Dietary therapy is less effective, but should be employed in B_6-unresponsive patients, especially in infancy where it is easiest to ensure compliance. A methionine-poor diet is usually supplemented with cysteine [4, 64], and plasma cystine is monitored to be within the normal range. Concentrations of homocyst[e]ine may also be reduced in B_6-unresponsive patients by treatment with betaine [65], but at the expense of raising methionine. Methionine should be kept below 1000 because of the risk of cerebral edema [23, 24]. Doses successfully employed have ranged from 50 to 200 mg/kg. The maximum licensed dose is 3 grams twice daily, the usual dose in adults. Especially rigorous therapy is necessary to ensure minimal levels of homocystine are maintained in preparation for surgery [4]. Vitamin C has been proposed to improve endothelial function [66]. Antiplatelet agents are used in patients with stroke to prevent recurrence [66]. A liver transplant has been performed in a B_6-unresponsive young adult with poorly controlled disease who had suffered multiple infarctions in the right cerebellum. Plasma and urine homocysteine and plasma methionine normalized after transplant without need of further dietary therapy or homocysteine lowering medications [67].

Ancillary supportive measures may be necessary. Orthopedic intervention may be required for pes planus and lower extremity valgus. The utility of agents such as bisphosphonates to increase bone mineralization remains to be established. Ectopia lentis may require aphakic contact lenses or spectacles; surgical intervention, such as lensectomy, may be indicated, and though there is controversy about the utility of implantation given the limited postoperative capsular support [68], intraocular lens implants may be considered.

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19

Maple syrup urine disease (branched-chain oxoaciduria)

MAJOR PHENOTYPIC EXPRESSION

Overwhelming illness in the first days of life with lethargy progressing to coma, opisthotonus, and convulsions, recurrent episodes leading to developmental delay, characteristic maple syrup odor, branched-chain amino acidemia, branched-chain oxoaciduria, deficiency of branched-chain oxoacid dehydrogenase.

INTRODUCTION

Maple syrup urine disease (MSUD) is a disorder of branched-chain amino acid metabolism in which elevated quantities of leucine, isoleucine, and valine and their corresponding oxo- and corresponding hydroxyacids accumulate in body fluids [1]. The disease was first described in 1954 by Menkes and colleagues [2], who observed an unusual odor quite like that of maple syrup in the urine of four infants who all died of a progressive encephalopathic disease in the first weeks of life. Elevated quantities of the branched-chain amino acids were found by Westall and colleagues [3], and the oxoacids derived from each of these amino acids were isolated and identified as their 2,4-dinitrophenylhydrazones by Menkes [4]. Defective decarboxylation of ^{14}C-oxoacid was demonstrated in leukocytes by Dancis and colleagues [5].

The fundamental defect is in the activity of the branched-chain oxoacid dehydrogenase multienzyme complex (Figures 19.1 and 19.2) [1, 6, 7]. The components are E1 (a decarboxylase), E2 (an acyl transferase), and E3 (a flavoprotein lipoamide dehydrogenase (dihydrolipoyl dehydrogenase)). E1 is composed of two proteins in an $\alpha_2\beta_2$ structure. The enzyme complex, which was purified to homogeneity by Pettit and colleagues [7], is analogous to the

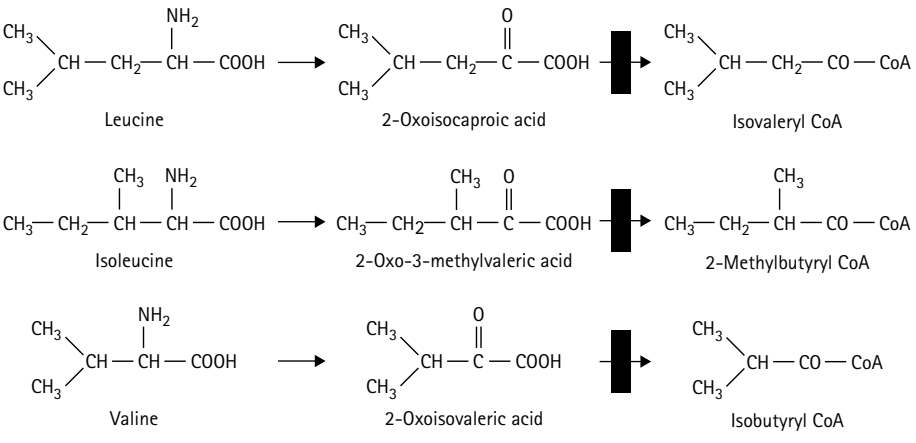

Figure 19.1 Metabolic pathways in the catabolism of leucine, isoleucine, and valine. The site of the defect is shown at the oxoacid step in each of the three pathways.

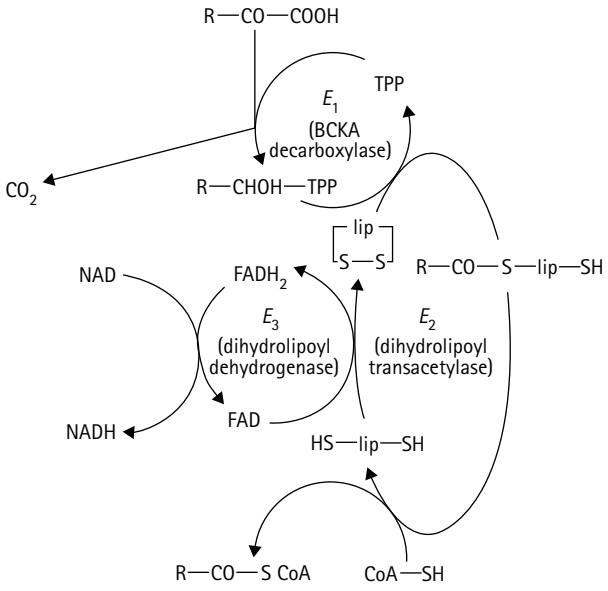

Figure 19.2 The branched-chain ketoacid dehydrogenase complex.

pyruvate and the 2-ketoglutarate dehydrogenase complexes; in fact, the E3 component of the three complexes is the same protein, and in E3 deficiency (Chapter 50) defective activity of each dehydrogenase enzyme results. Expression studies have shown that the complex does not assemble spontaneously; the E1 α and β proteins require chaperonins for folding and assembly [8].

The cDNA of each of the component genes has been cloned. The E1α gene has been localized to chromosome 19q13.1-13.2 [9], E1β to 6p21-22 [10], E2 to 1p31 [10], and E3 to 7q31-32 [11]. Mutations have been identified in each gene. To date, a majority has been in the E1α and E2 genes. The mutation in the Mennonite population in which MSUD is common is a T to A transition that yields a single missense tyrosine to asparagine (Y393N) change at position 393 of the E1α gene [12]. In the Ashkenazi Jewish population, p.R183P mutation in the E1β gene has been found in high frequency [13].

CLINICAL ABNORMALITIES

Infants with classic MSUD appear normal at birth, but they usually remain well for only a few days. Pathologic amino acids rise by 12–24 hours and irritability, vomiting or difficulty to feed may be early symptoms at age two to three days. Usually by four to five days they become lethargic and progressive neurologic deterioration is rapid [14–16]. The cry may be high pitched. There may be intermittent apnea, stereotypical movements such as "fencing" and "bicycling" and periods of flaccidity, in which deep tendon reflexes and Moro reflex are absent, alternating with hypertonicity. General muscular rigidity is common. The absolutely characteristic picture is of a markedly comatose

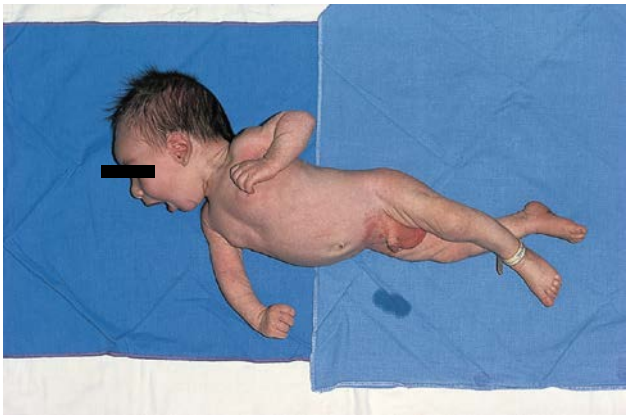

Figure 19.3 MB: An 11-day-old infant with maple syrup urine disease. He is shown in the characteristic opisthotonic position. (The illustration was kindly provided by Dr Havelock Thompson of the University of West Virginia.)

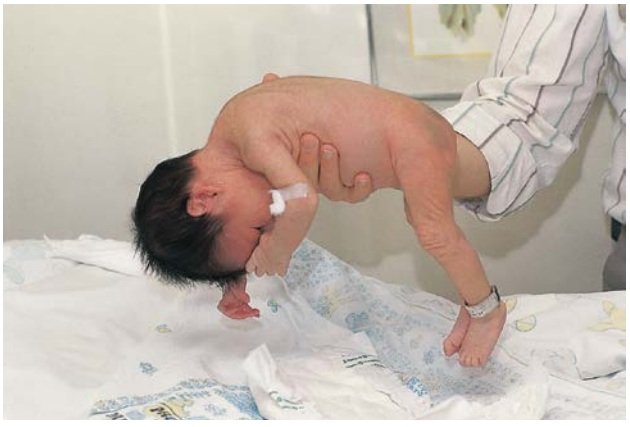

Figure 19.4 GV: A 3-week-old infant with maple syrup urine disease who presented with almost pure hypotonia. At this age, treatment had begun and tone improved somewhat, but he was still largely flaccid and had no sucking reflex. Tone and sucking became normal as the levels of the branched-chain amino acids were brought to normal.

or semicomatose infant with hypertonia in an opisthotonic position (Figure 19.3). Extreme opisthotonus of this degree is very unusual in an infant only a few days old. There may be dystonic extension of the arms or a decerebrate appearance. Rarely, an infant may present with hypotonia and flaccidity (Figure 19.4). There may be abnormal eye movements. Convulsions occur regularly. These symptoms proceed to apnea, coma, and death by age seven to ten days, unless a vigorous therapeutic program is instituted [14]. Cerebral edema and a picture of pseudotumor cerebri may be seen and has been documented by computed tomography (CT) or magnetic resonance imaging (MRI) scan [17–20].

Rarely, an infant who is not effectively treated may survive this early phase of the disease but will always be left with prominent neurologic abnormalities and severely impaired mental development (Figures 19.5–19.9). Any

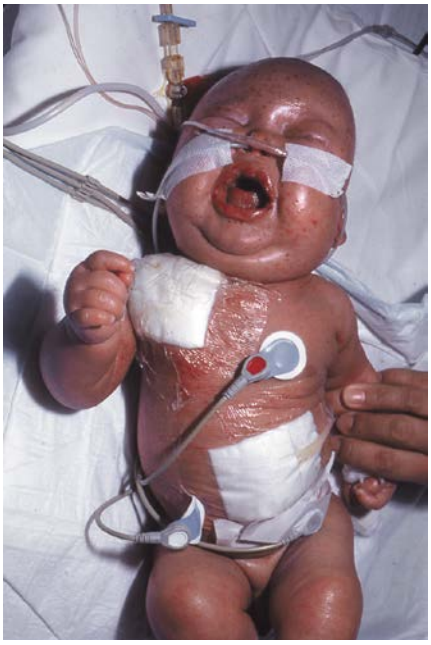

Figure 19.5 A Saudi patient who was admitted in the first week of life with coma and convulsions, and was found to have maple syrup urine disease. He required ventilation, peritoneal dialysis, and parenteral nutrition. He spent four months in hospital and was discharged in good condition.

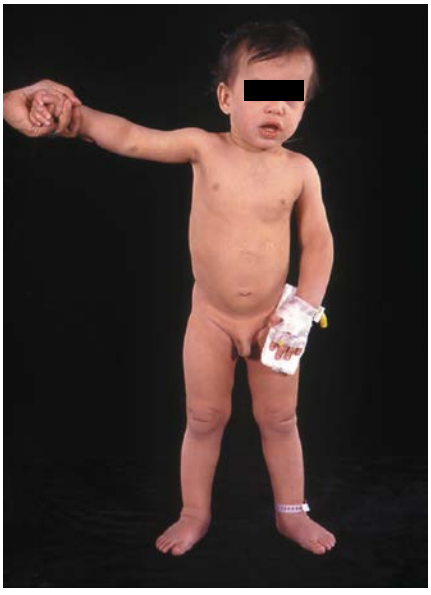

Figure 19.6 The same patient at two years of age. He had normal growth; however, his development was at the 18-month level.

patient with MSUD remains a candidate for further episodes of acute overwhelming illness and coma, any one of which may be fatal or lead to neurologic damage. Episodic illness is often triggered by the catabolic state that accompanies infection. It may also follow dietary indiscretion in which the amount of protein ingested by normal infants or children is consumed. Cerebral edema and death have

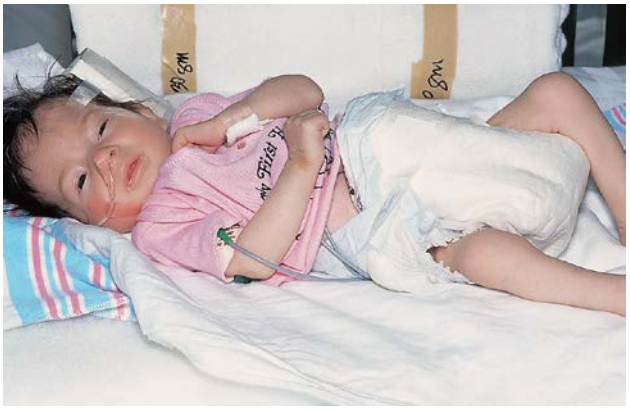

Figure 19.7 Mexican infant with maple syrup urine disease in relapse. She was semicomatose, had hypertonia, and had exaggerated deep tendon reflexes and ankle clonus.

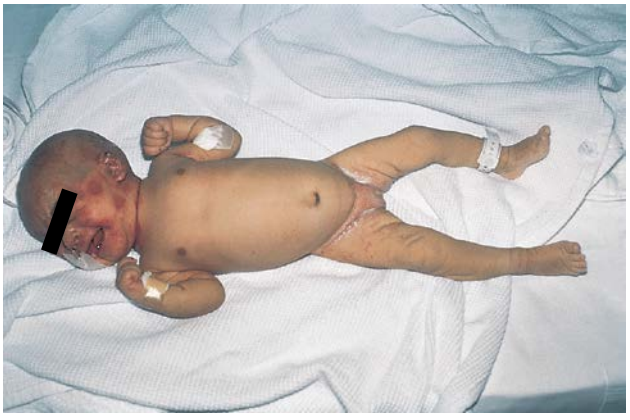

Figure 19.8 ESH: A Saudi infant with maple syrup urine disease. He was quite rigid. The dermatitis reflects the problem of the dietary management of the disease.

been reported in four infants between three and five years of age during therapy for severe metabolic acidosis and dehydration, which occurred with intercurrent infection [18]. Four patients with cerebral edema were documented to have hyponatremia and decreased osmolarity in the serum [20]. It is absolutely critical that electrolytes are checked every 6 hours during emergency treatment, and serum sodium should be maintained at ≥138 mmol/l.

The characteristic odor may be detected as soon as neurologic symptoms develop. At the same time, it should be pointed out that not every patient with this disease is recognizable by the smell. Infants with this clinical picture should be screened for metabolic disease, whether or not an odor is detected. The odor is particularly likely to be absent in a comatose patient who has not received protein for days and has received copious amounts of parenteral fluid prior to transfer from a referring hospital. The odor may be found in the hair, the sweat, or cerumen. It is usually best appreciated in the urine. Freezing the urine may bring out the smell by concentrating it in an oil that freezes poorly or not at all at the top of the frozen specimen

Figure 19.9 CH: A teenager with maple syrup urine disease. She has severely impaired mental development and ataxia.

Figure 19.10 Frozen urine from a patient with untreated maple syrup urine disease. The odor of maple syrup is concentrated in an oil at the top.

(Figure 19.10). The odor is sweet, malty, or caramel-like. It really does call forth an olfactory image of maple syrup. The odor of the patient with the disease once appeared provincially North American because of the localized occurrence of maple syrup. Then Mediterraneans and others [21–23] realized that this odor was produced by the ingestion of fenugreek (*foenum graecum*) from Trigonella by an infant, or by the mother prior to delivery. In Central Europe, the smell is found in a common fluid spice called Maggi®. The compound responsible for the odor was isolated and identified as sotolone (4,5-dimethyl-3-hydroxy-2[5H]-furanone). This compound, derived as a cyclization product of isoleucine, has now been isolated from the urine of patients with MSUD, as well as from maple syrup itself [24].

In contrast to the classic organic acidurias, there is no accumulation of CoA metabolites (and thus no characteristic acylcarnitines), and acidosis or hyperammonemia are not major features, though the patient may be ketonuric. Hypoglycemia may be observed in the acute episode of illness, but is not common [25, 26]. Pancreatitis has been observed in MSUD, as it has in other organic acidemias [27, 28]. One patient with pancreatitis had transitory retinopathy [26].

The electroencephalogram (EEG) of the newborn with MSUD has been described as a comb-like rhythm (5–9 Hz) of spindle-like sharp waves over the central regions and multiple shifting spikes and sharp waves with suppression bursts [28, 29]. Later, the EEG may be normal between attacks, when there may be generalized slowing or paroxysmal discharges. A normal EEG may be seen despite abnormalities on CT of the brain and developmental delay [30]. Leucine loading in an asymptomatic adult with MSUD led to EEG abnormalities [31]. During acute metabolic decompensation, patients with MSUD may develop acute sensor-motor polyneuropathy, which can persist for months [32].

Neuroimaging most commonly shows decreased attenuation in white matter consistent with delayed or abnormal myelination [16, 29, 33]. This appearance resolves after some months of successful treatment. In one patient [33], complete resolution of white matter lucency on CT of the brain was seen after 40 days of treatment. Impressively, ventricular size decreased. Generalized lucency of the cerebral white matter has been seen as early as nine days, despite a restricted diet [34]. In nine of ten patients with classic MSUD, who had general lucency, there was also localized intense lucency in the deep cerebellar white matter and peduncles and the brain stem. These changes have been attributed to edema [34], but may well be dysmyelination or delayed myelination [26]. In one of our patients, MRI at one month showed striking lucency of the white matter which had markedly improved by one year of age. In another patient, studied first by MRI of the brain at six years of age, in whom the initial diagnosis had been late, the MRI was normal, except for a slight increase in ventricular size.

The usual neuropathologic finding in patients dying of MSUD is a generalized status spongiosus of the white matter similar to that seen in phenylketonuria and nonketotic hyperglycinemia [35]. The changes have generally been described in infants nine months to 4.5 years of age, but spongiform change was reported along with edema in an infant who died at 12 days of life [36]. Patients in the original series [2] who died at 11 and 14 days had cerebral edema. Spongiform changes and intramyelin vacuoles

on electron microscopy have been observed in an animal model in which Hereford calves died of MSUD within the first week of life [37]. In older children and adults, symptoms of metabolic decompensation are more variable, including acute or semiacute cognitive impairment, hyperactivity, sleep disturbances, hallucinations, mood swings, focal dystonia, choreoathetosis, and/or ataxia. Without early presymtomatic detection and treatment, 70–90 percent of surviving older patients have impaired global function and intelligence, anxiety disorders and depression [38] as well as movement disorders (tremor, dystonia, parkinsonism, pyramidal signs and a spastic-dystonic gait) [39]. In our experience, this is fortunately very different in children diagnosed by newborn screening and treated early [40].

A number of variants of branched-chain oxoaciduria has been described in addition to the classic, severe, or neonatal form [41]. Each is milder in its clinical presentation than classic MSUD. The first of these to be described [42–45] and the second most common form has been referred to as intermittent branched-chain ketoaciduria. Involved individuals may have no problems except in the presence of some special stress, such as infection or surgery. On the other hand, this disorder too can be lethal. Patients with no symptoms at all for a period of years can suddenly develop coma, convulsions, and death following an apparently mild infection [45]. More commonly, these patients have intermittent bouts of acute ataxia. In one report, severe acidosis was a prominent clinical finding [44]. In previous editions of this book, we have cited our experience that the biochemical abnormality of the accumulation of amino acids and oxoacids in body fluids is not intermittent, even though the clinical manifestations may be. In all of these patients, the biochemical features were always demonstrable, except of course when successfully treated. We have now encountered patients who clearly fit the definition of intermittent even of the biochemistry.

A third form has been referred to as intermediate branched-chain ketoaciduria [46, 47]. These patients usually presented with mental impairment and hence some symptomatology was considered to be continuous as opposed to intermittent. Unfortunately, such a clinical course also occurs due to inadequate treatment, especially overtreatment, when protein restriction is continued too long at minor intercurrent illnesses and especially long-term. Protein deficiency induces catabolism and, in its extreme, life-threatening form manifests as dermatitis (see Figure 19.8), alopecia and diarrhea, an acrodermatitis enteropathica-like syndrome unrelated to zinc deficiency. If plasma concentrations of isoleucine and valine are below normal for prolonged periods, growth and development will become retarded or seize and often never catch up completely even if therapy is later optimized. After detection of patients through newborn screening months-long amino acid imbalances maybe more deleterious for adequate outcome than acute decompensations. One patient reported as intermediate [47] presented first with ketoacidosis and coma at the age of ten months and subsequently responded to dietary therapy with no further episodes and had an IQ of 92. Some have presented with opthalmoplegia [48, 49]. Nevertheless, it is increasingly clear that what we are dealing with is a continuum. Dancis [41] based a classification of variants on protein intolerance. In the classic form of the disease, he considered the patients unable to tolerate maintenance requirements of protein and requiring artificial purified amino acid diets for survival. Enzyme levels were 0–2 percent of normal. In the second group, protein tolerance was sufficient to maintain normal growth in infancy or 1.5–2.0 g/kg of protein. Enzyme levels in this group were between 2 and 8 percent of normal. In the third group, an unrestricted diet was tolerated. Enzyme activity was between 8 and 16 percent of normal.

Oxidation of labeled leucine *in vivo* served as a much better discriminant than *in vitro* enzyme activity of variant forms of MSUD [50]. In patients with classical infantile disease, it was unmeasurable. In severe variants, it was 4 percent of control. In six milder variants, it ranged from 19 to 86 percent of control.

Another variant may be distinguished by the fact that the biochemical abnormalities are corrected by the administration of high doses of thiamine [51]. This thiamine-responsive MSUD or thiamine-responsive branched-chain oxoaciduria was originally described in a patient with relatively mild clinical symptomatology who responded to as little as 10 mg of thiamine per day [51]. Patients described to date have all had residual activity of the enzyme [52, 53], but doses up to 300 mg/day have been required, and two of them presented with classical clinical disease in infancy. These patients have been quite heterogeneous and nutritional therapy has been necessary.

A patient with E3 deficiency [54] presented with feeding difficulties in the first week, vomiting, and failure to thrive. By six weeks, severe developmental delay was apparent along with hypotonia and very poor head control. At eight months, he had lactic acidosis (10 mmol/L) and respiratory distress. He died in severe acidosis following liver biopsy at 18 months of age. E3 deficiency is considered in Chapter 50.

GENETICS AND PATHOGENESIS

MSUD is transmitted as an autosomal recessive trait. This is true of each of the variants. Classic MSUD has been seen throughout the world and in all ethnic groups. It is common among the Mennonites of Pennsylvania in whom the incidence is one in 400 [20]. In the German screening program, an incidence of one in 165,000 was encountered [40]. Heterozygote detection by enzymatic assay may not be reliable. Once a mutation is identified in a proband, molecular techniques may be used to establish carrier status. The activity of the enzyme can be measured in cultured amniotic fluid cells, and the disease has been diagnosed prenatally in a substantial number of patients.

Mutations can readily be tested for in prenatal diagnosis, especially with allele-specific oligonucleotide probes [12].

The molecular defect in MSUD is in the branched-chain oxoacid dehydrogenase which catalyzes the decarboxylation of the oxoacids (see Figures 19.1 and 19.2). Activity is widely distributed in mammalian tissues. The enzyme is located on the inner surface of the inner mitochondrial membrane. Activity can be measured in the human liver, kidney, and leukocytes, cultured fibroblasts or lymphoblasts, and amniotic fluid cells [41, 55–57].

In the reaction, as in the case of pyruvate or α-ketoglutarate, there is first a thiamine pyrophosphate (TPP)-mediated conversion of the carboxyl group to CO_2 and the formation of a covalently bound enzyme, TPP, substrate complex (Figure 19.2). Next, there is an oxidative transfer to the second, lipoic acid-bearing enzyme, liberating TPP after which there is transfer to coenzyme A, and lipoic acid is regenerated. Regulation of enzyme involves acylCoA compounds, and activity is stimulated by carnitine [58], presumably by the formation of acylcarnitine esters of acylCoA compounds and prevention of product inhibition. The enzyme is inhibited by adenosinediphosphate (ADP), a condition under which pyruvate decarboxylation is stimulated. Additional regulation has been demonstrated in a phosphorylation/dephosphorylation cycle in which the dehydrogenase complex is inactivated by the kinase BCKDK catalyzed phosphorylation and activated through action of the phosphatase. Recently, mutations in BCKDK were identified in patients with very low branched-chain amino acids and clinical symptoms of autism, epilepsy, and intellectual disability that may respond to dietary treatment [59].

Measurement of the activity of branched-chain ketoacid decarboxylase *in vitro* in fibroblasts or leukocytes has generally been carried out by studying the conversion of leucine-^{14}C, isoleucine-^{14}C, valine-^{14}C, or α-oxoisocaproic acid-^{14}C to $^{14}CO_2$. In patients with classic MSUD, each activity has been virtually nil [6, 60]. In contrast, the oxidation of isovaleric acid-^{14}C to $^{14}CO_2$ is normal.

Patients with intermittent branched-chain oxoaciduria and other variants have been found to have residual activity [6, 41]. Activity of up to 15–25 percent of the normal level has been observed [47]. A patient with E3 deficiency had defective activity of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase, as well as BKAD [54].

Abnormal activities have been identified in the individual components of the enzyme complex [61], but these assays are demanding, and dissection of the individual components in patients has been facilitated by northern and western blotting with specific antibodies and cDNA probes [61, 62]. This dissection has been accomplished by retroviral complementation of dehydrogenase activity using plasmids containing the wild type for each of the three genes E1α, E1β, and E2; the one that restored activity identified the mutated gene [63]. There may be functional deficiency of E1β and lack of immunoreactive protein as a consequence of mutation in E1α [12], consistent with a requirement for protein interactions in assembly and stabilization.

Mutational analysis has identified more than 150 disease-causing mutations in the branched-chain α-ketoacid dehydrogenase multienzyme complex, most in E1α [62–70].

The vast majority of these have been missense mutations and most have been associated with the severe, classic phenotype. The most prevalent mutation, the T to A change found in Mennonites [12], has also been found in other populations [65]. Of three other missense mutations in E1α, one led to a classical phenotype [65], and two were intermediate in Mexican-American patients [66]. The G245R mutation appears to be common in that population. Four E1α mutations are common in Japanese [67].

A founder effect and mutational hot-spot mutation has been reported c.11/del C in Portuguese gypsies in whom MSUD is common [68]. A patient detected by newborn screening was found to be developing normally and passed rigorous psychometric assessment despite the presence of the characteristic biochemical phenotype, including alloisoleucine. He was found to have a novel intronic change c.288+9C>T in the *BCKDHA* gene on one allele and p.G249S, a mutation previously associated with severe phenotype on the other [69]. He was found to have variably spliced products, including some normal mRNA. In the E1β gene, many mutations were found in Japanese [67]. An 11 base-pair deletion in the mitochondrial target sequence is relatively common [70]. Five mutations were found [68] in Portuguese, three of them nonsense, p.P200X, Q267Y, and R285X.

A number of mutations have been found in E2 [63, 71–75]. They include single-base substitutions [70–73], insertions [66, 72], and deletions [72, 75], and these mutations have led to missense, nonsense, frameshift, and internal deletion, as well as exon skipping at splice junctions and coding regions. Many have been compounds of two mutations [71–73]. Three novel missense mutations and a frameshift were found in Portuguese patients [68]. The E2 gene appears to have a propensity for splicing errors, some induced by large mutations in introns. The original thiamine-responsive patient of Scriver *et al.* [51] had a 17 base-pair insertion in the E2 mRNA [71], resulting from a deletion in intron 4 [74]. Many of the E2 variants have been seen in patients with clinical variant phenotypes.

Five missense mutations have been reported in E3 in a single-compound patient [74, 76]. A G229C mutation is common in Ashkenazi Jews [76].

Defective activity of the dehydrogenase complex leads to elevated concentrations of leucine, isoleucine, and valine in the plasma and urine (Table 19.1) [3, 15, 16, 74]. Racemization of the 3-carbon of L-isoleucine during

Table 19.1 Concentrations of amino acids in plasma in untreated maple syrup urine disease

Valine (μmol/L)	Isoleucine (μmol/L)	Leucine (μmol/L)	Alloisoleucine (μmol/L)
500–1800	200–1300	500–5000	Trace–300

Table 19.2 Organic acids of the urine in maple syrup urine disease

Oxo acids (mmol/mol creatinine)			Hydroxy acids (mmol/mol creatinine)		
2-Oxoisocaproic acid	2-Oxo-3-methylvaleric acid	2-Oxoisovaleric acid	2-Hydroxy-isocaproic acid	2-Hydroxy-3-methylvaleric acid	2-Hydroxy-isovaleric acid
400–4400	500–2500	600–800	3–8	60–400	850–3600

transamination results in the formation of L-alloisoleucine. Its elevation is pathognomonic for MSUD [77].

Patients also excrete the oxoacid products of the transamination of each of these amino acids (Table 19.2), consistent with the site of enzymatic block (see Figure 19.1) [15, 16, 74]. Isovaleric acid, α-methylbutyric acid, and isobutyric acid are not found. Among the amino acids, the concentration of leucine is virtually always higher than those of isoleucine and valine [3]. An exception was a variant patient described as valine-toxic [78]. Two other patients were unusual in that most of the branched chain oxoacids were derived from isoleucine [79, 80]. The oxoacid analogs of leucine and isoleucine are usually present in much higher quantities than the corresponding hydroxyacids. In contrast, 2-hydroxyisovaleric acid is usually present in much higher concentration than its oxoacid. Alloisoleucine is also regularly found [77]. This product of isoleucine accumulation was originally mistaken for methionine in the amino acid analyzer, creating some confusion in the management of early patients. The concentration of alanine in the plasma of these patients is decreased [14]. During illness as the leucine rises, the alanine falls. The molar ratio of leucine to alanine is a more sensitive measure than leucine alone, and may be useful in diagnosis or newborn screening [20]. In a series of 18 newborns with MSUD, the ratio was 1.3 to 12.4 (normal, 0.12–0.53).

Screening for the disease has been carried out by the addition of 2, 4-dinitrophenylhydrazine, which produces a yellow precipitate of dinitrophenylhydrazones [81] in the presence of oxoacids. The individual oxoacids have been distinguished by gas chromatography-mass spectrometry (GCMS) of the oximes [82]. The ferric chloride test on the urine may yield a greenish-gray color.

Rapid screening for MSUD is now done best by tandem mass spectrometry (MS/MS), and this forms the basis for all of the neonatal screening programs for this disease [40]. The method is not useful for management because it does not distinguish between leucine and isoleucine. This is not a problem for screening, but we have encountered treatment failure and maltreatment when this method has been used for monitoring of therapy. Actually, hydroxyproline and alloisoleucine are isobaric with leucine and isoleucine; so newborn screening does not separate any of them. A positive screen is followed up by plasma amino acid analysis, which distinguishes all of these and rules out hydroxyprolinemia, the genetic cause and prevalence of which have recently been delineated. Hydroxyprolinemia is a nondisease due to homozygous or compound heterozygous mutations in *PRODH2* and an estimated prevalence of about one in 50,000 newborns [83], i.e., at least three times as common as MSUD. A liquid chromatography mass spectrometry (LC-MS/MS) method has been developed for alloisoleucine which serves as a second-tier test for newborn screening of dried blood spots [84]. The leucine (isoleucine) alanine ratio [20] should be a useful assessment in screening programs.

TREATMENT

Emergency treatment of an infant in coma requires prompt reduction of levels of leucine and the other branched-chain amino acids. This has formerly been approached by exchange transfusions, peritoneal dialysis, or both; but direct measurements have indicated the removal of small quantities of amino acids in this way. Continuous arteriovenous or venovenous hemodialysis, hemofiltration and hemodiafiltration are doubtless very effective [85, 86], but it is formidable in a young infant, and the prospect of repeat dialysis with each respiratory infection in the early years of life is impossible to consider. Saudubray and colleagues [85, 86] have reported on continuous venovenous extracorporeal hemodiafiltration as a very effective method for the lowering of high levels of leucine. In six neonatal infants and six children with later episodes, this approach was begun after six hours of conservative management including enteral amino acid mixtures, and leucine concentration, was over 1700 μmol/L. In each, the decrease in leucine was logarithmic and usually reached $<$1000 μmol/L in 24 hours, while the rate of decrease with enteral therapy after cessation of the diafiltration was a slower linear fall. Follow-up developmental levels in this series were encouraging; some had intelligence quotients over 100 with follow up of as long as three to five years.

The alternative approach has been to take advantage of the power of the anabolic laying down of accumulated amino acids into protein to lower toxic levels of branched-chain amino acids. This can be accomplished by providing mixtures of amino acids not containing leucine, isoleucine, and valine and energy as provided by 10 percent glucose intravenously. Intravenous solutions have been developed for this purpose and shown to be rapidly effective [87–89], but these solutions are not generally available, and are very expensive. We have successfully employed 200 mL/kg of 10 percent glucose intravenously and 2 g/kg of amino acid mixture providing 88 cal/kg. Berry and colleagues [88] have used larger quantities of glucose, requiring a central line and insulin. In a patient who is not vomiting, it is also possible to accomplish this by intragastric drip.

Table 19.3 Management of the acute crisis in maple syrup urine disease

Stop oral feedings. Begin intravenous hydration with 10% glucose and maintenance electrolyte.
Begin nasogastric or gastrostomy drip with 2.0–4.0 g/kg of amino acid protein equivalent – lacking leucine, isoleucine, and valine.
Complex MSUD Amino Acid Blend: 13 g of mixture provides 10 g of amino acids — 2.0 g/kg = 2.6 g/kg of complex. This contains 8 cal/kg. Add H_2O to make 8 mL/kg or 1 cal/mL to make for minimal volume in a vomiting or potentially vomiting patient, and drip this volume in slowly over 24 hours.
Obtain plasma stat for amino acids at least every 12 hours initially until therapeutic trend is established; thereafter, at least daily.
Plan to add isoleucine and valine even in the first 24 hours, as hypoisoleucinemia will stop anabolism, lead to catabolism and consequent rise in leucine. A level of 20 mg/kg of added isoleucine is usually sufficient, but if added later even 100 mg/kg may be required. Valine supplementation may also be necessary before a steady-state leucine level is achieved.
In patients needing additional therapy, add insulin (0.1 U/kg per hour). Provide intravenous glucose as 10% – at least 20 mL/U insulin. Monitor blood sugar and urine – dipstix and adjust.
In patients needing additional therapy, add s.c. human growth hormone 0.05 mg/kg per 24 hours.
Thiamine at 100 mg/kg can be given parenterally at the start of therapy. Patient could be discharged with p.o. allithiamine and later tested to see if thiamine added anything to treatment.

Even in the presence of vomiting, we have found that provision of enteral amino acid mixtures dripped in minimal volume over 24 hours are usually tolerated [90, 91]. Our mixtures contained extra quantities of alanine and glutamine [91] and so do those employed by Morton *et al.* [20]. In their extensive experience, the rate of fall of plasma leucine with this approach was consistently greater than those reported for dialysis or hemoperfusion [20]. Commercial mixtures suitable for enteral use in minimal volume for the management of the acute episodes are now available in the United States (Complex® MSUD Amino Acid Blend; Applied Nutrition, Randolph, NJ, USA). A protocol for acute management is given in Table 19.3. The table provides an approach to the prevention of deficiencies of isoleucine and/or valine that regularly occurs during leucine reduction, because there is virtually always more leucine than the other branched-chain amino acids. Deficiency of either of these essential amino acids leads to catabolism and long-term to breakdown of the skin (Figure 19.11). For some years, we and others [20] have added isoleucine and valine from the beginning of emergency treatment including supraphysiological levels (300–500 μmol/L).

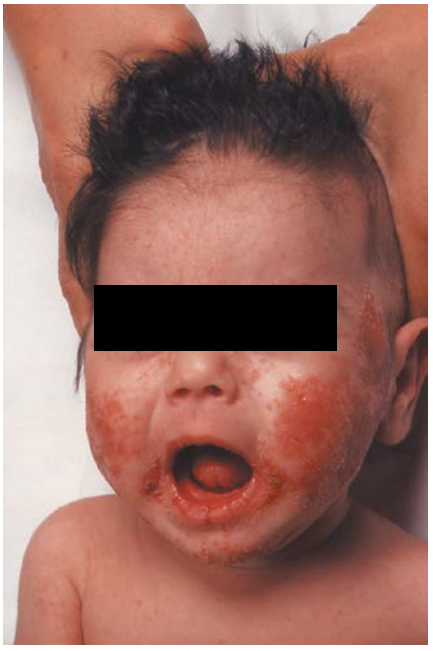

Figure 19.11 A six-month-old Saudi patient with maple syrup urine disease, who developed isoleucine/valine deficiency and its skin manifestations that often complicate the treatment of this disease. Cutaneous manifestations receded after supplementation with isoleucine 10 mg/kg per day and valine 60 mg/kg per day.

Wendel and colleagues [92] treated the acute crisis with insulin and glucose as an anabolic approach to therapy. In each episode studied, the introduction of this regime led to reduction in toxic levels of leucine. Studies of the *in vivo* metabolism of ^{13}C-leucine have indicated that protein synthesis is normal, and that there is no significant route for disposal of leucine other than protein synthesis [93], providing further argument for anabolic approaches to therapy. In these studies, leucine oxidation was undetectable, consistent with what we have found in fibroblasts *in vitro* [94]. In our experience, enteral anabolic therapy, parenteral insulin, and glucose along with human growth hormone were always successful and sufficient (Table 19.3).

Chronic management consists of restricting the intake of each of the three branched-chain amino acids to those essential for growth and no more. This type of dietary management is much more difficult than that of phenylketonuria. It requires very close regulation of an artificial diet and frequent access to an amino acid analyzer (Table 19.4). During rapid growth velocity (0 to 10 months), small infants usually require and tolerate 50–90, 30–60, and 20–50 mg/kg per day of L-leucine, L-valine, and

Table 19.4 Optimal therapeutic ranges of branched-chain amino acids in plasma in MSUD

Leucine (μmol/L)	Isoleucine (μmol/L)	Valine (μmol/L)
100–200	50–150	150–250

L-isoleucine, respectively. These amounts will gradually decrease. After one year of age, requirements for L-leucine, L-valine, and L-isoleucine usually range around 20–40, 10–40, and 5–20 mg/kg per day. Thereafter, the tolerance of leucine decreases further with age down to 5–15 mg/kg per day in adults, whereas the requirements for L-valine, and L-isoleucine usually change only a little. The best results are seen in those in whom treatment has been initiated earliest. The largest experience with the management of this disease is that of Snyderman *et al.* [14, 95] and Morton *et al.* [20], and both have written that there can be little doubt about the beneficial effect of therapy in this disorder. Commercial products are available that are useful in the management of this disorder (Ketonex-Ross, MSUD-Mead-Johnson) [96].

There are no good protocols to test for thiamine-responsiveness. In thiamine-responsive MSUD, the doses employed have ranged from 10 to 300 mg/day [51, 53]. It has appeared reasonable to test each patient with larger amounts before deciding that thiamine is not a useful adjunct to therapy. However, this is complicated by data that indicate not more than a few milligrams of an oral dose are absorbed [97], implying that parenteral administration may be necessary to assess the effects of larger doses. Allithiamine may be useful orally.

The management of intercurrent illness is particularly important in this disease [98], and parents must be taught to be efficient partners in recognition and prompt management. Written protocols or letters to Emergency Room physicians for use when illness occurs in out of town situations are useful adjuncts. A regimen of restriction of leucine intake at the first sign of illness, continuation of other amino acids (including 10 mg/kg of isoleucine and of valine) and the supply of abundant calories particularly as glucose or glucose polymer is useful.

Liver transplantation is an option in the treatment of MSUD [99–104]. Orthotopic liver transplantation was carried out in two patients with the disease for nonmetabolic reasons. Both had liver failure, one from hepatitis A [99] and the other from intoxication with vitamin A [100]. Both have remained metabolically and neurologically stable without any restriction of protein intake for over two years. A third patient [5, 101] was transplanted because of the request of parents concerned with delayed psychomotor development and frequent metabolic decompensation. In each patient, dramatic decrease in plasma levels of branched-chain amino acids occurred immediately, reaching near normal levels in 10 hours despite post-transplant catabolic stress. Whole-body stable isotope-labeled leucine oxidation was normal in the one patient tested. Another patient has received a liver transplant because of liver failure [101]. At nine years, neurologic findings included stupor, dystonia, ataxia, hyperreflexian, and positive Babinski signs. Five years following the procedure, neurologic examination was normal as were plasma amino acid concentrations and calculated brain uptake of neutral amino acids.

Domino hepatic transplantation was reported [101, 102] in a 25-year-old man with MSUD who had had a number of recent admissions to hospital for metabolic imbalance. He had none post-transplantation. His liver was given to a 53-year-old man with cancer who otherwise would not have qualified for a liver. Both patients remained metabolically stable while receiving completely normal intakes of protein. Studies of ^{13}C-leucine oxidation to expired $^{13}C0_2$ revealed both patients to have levels intermediate between their pretransplant levels. Neither had appreciable alloisoleucine.

A major experience with hepatic transplantation in MSUD was published [103] representing a collaboration between the Clinic for Special Children, Strasburg, PA where MSUD is common and the transplant center at the University of Pittsburgh. Eleven patients were reported. All were alive and well at report after 106 months in the index patient and 4–18 months (median 14 months) in the subsequent ten. Leucine levels were stable on an unrestricted diet and patients remained stable during protein loading and intercurrent illness. In summary, none of all transplanted patients suffered a further episode of metabolic derangement until recently acute metabolic crises even requiring dialysis were reported in a toddler after successful liver transplantation from his mother [106].

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20

Branched chain keto acid dehydrogenase kinase (BCKDK) deficiency

MAJOR PHENOTYPIC EXPRESSION

Severe mental retardation, autism, seizures, hypotonia, microcephaly, cerebral atrophy, very low concentrations of leucine, isoleucine and valine in plasma and cerebrospinal fluid (CSF) and decreased activity of BCKDK.

INTRODUCTION

Deficiency of BCKDK has been reported in six patients with very severe deficiency of motor and cognitive function, autism, seizures and cerebral atrophy [1, 2]. The kinase enzyme is responsible for phosphorylating the enzyme branched chain keto acid dehydrogenase (Figure 20.1). This phosphorylation downregulates the activity of the enzyme; so, its deficiency leads to very robust dehydrogenase activity. This increased oxidation of the branched chain amino acids leads to very low concentrations of the branched chain amino acid leucine isoleucine and valine, in the blood and CSF fluid. Plasma concentrations reported have been under 20 μmol/L. The activity of the enzyme has been found to be defective. Mutations reported have included p.R174Gfs and on the other allele p.L389P [2] and homozygosity for pM74fs, p.R156X and p.R224P [1]. Some improvement has been reported after treatment designed to increase

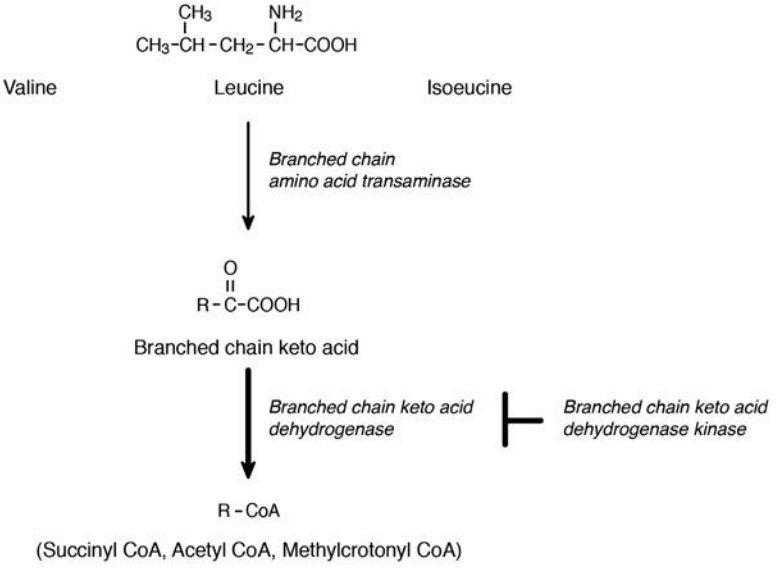

Figure 20.1 Schematic pathway of branched chain amino acid catabolism and mechanism for the reduction of branched chain amino acid concentration.

levels of the branched chain amino acids, and neurologic abnormalities were abolished in an animal model within a week of starting a diet enriched with branched chain amino acids.

CLINICAL ABNORMALITIES

Early symptoms in two patients reported [2] were those of seizures and developmental delay. Both were hypotonic. Microcephaly was soon apparent. Physical growth was also retarded. By the age of four years, one patient had a developmental age of 12 months. The other at five years of age functioned at the 39-month level. Seizures were accompanied by spike discharges on the electroencephalogram (EEG), and magnetic resonance imaging (MRI) of the brain displayed considerable diffuse loss of cerebral substance. Both patients had autistic traits. In the other series [1], consanguineous families with autism, and intellectual disability either had seizures or abnormalities in the EEG. Amino acid analysis revealed very low concentrations of all of the branched chain amino acids in blood plasma and in CSF. Concentrations of leucine in the blood range from 12 to 17 μmol/L and that of isoleucine from 4 to 14 μmol/L.

GENETICS AND PATHOGENESIS

The disease is autosomal recessive. Consanguinity has been prominent in the families reported [1].

Enzyme assay has documented deficiency of the activity of BCKD enzyme in fibroblasts.

Mutations reported to date in the gene on chromosome 16 have included the null mutation R174G and a missense mutation p.L389P [2]. A C to T change in exon 4 led to a premature stop at position 156 [1], prior to the kinase domain. A single-base deletion c.G222 del in exon 2 led to a frameshift that terminated the protein at position 74 of R412 amino acids [1]. A missense mutation G671C led to a change from a highly conserved arginine at 224 to a proline. Levels of mRNA were shown to be reduced in two families studied [1], suggesting nonsense mediated decay, and enzyme protein was undetectable by western blot [1].

Mice with deficiency of BCKDK have increased basal activity of the BCKDH complex [3]. These mice developed neurologic abnormalities including seizures; histology of the brain was normal.

Branched chain amino acids are transported across the blood–brain barrier by the amino acid L-type transporter (LAT1) pathway shared by other large neutral amino acids. In the mice, levels of the branched chain amino acids were low in brain. Levels of threonine, phenylalanine, tyrosine, histidine, and methionine were high.

TREATMENT

It appears logical to attempt to raise levels of the branched chain amino acids. It has been approached by a marked increase in the intake of protein to 3.5 g/kg of protein in foods, along with 110 mg/kg of each of the amino acids every five hours. Continuous intragastric feeding has been continued overnight [2].

This regimen has led to major improvement to normal in the levels of these amino acids in the plasma. There have also been some clinical improvements, including attaining the ability to walk. Growth was noticeably improved after six months of treatment. In the mouse model [1], animals raised on diets enriched in branched chain amino acids were phenotypically normal, and animals without the enrichment had seizures and neurologic abnormalities that could be abolished by enrichment of the diet.

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21

Oculocutaneous tyrosinemia/tyrosine aminotransferase deficiency

MAJOR PHENOTYPIC EXPRESSION

Dendritic keratitis, causing lacrimation, photophobia, inflammation, ulcers, and scars; keratoses of the palms and soles; hypertyrosinemia; defective activity of hepatic cytoplasmic tyrosine aminotransferase.

INTRODUCTION

Oculocutaneous tyrosinemia was first described in 1967 by Campbell and colleagues [1] in a report of a patient with corneal ulcers, erythematous papular lesions on the palms and soles, and severe impairment of mental development. A number of patients have since been reported, and it is clear that impaired mental development is not a uniform feature of the disease [2–8]. Patients were described in 1938 by Richner [9] and in 1947 by Hanhart [10] with typical lesions of the eyes and skin, and this came to be known as the Richner–Hanhart syndrome, or keratosis palmaris et plantaris. It appears likely that oculocutaneous tyrosinemia and the Richner–Hanhart syndrome are the same disease, although plasma concentrations of tyrosine are not available for the original patients of Richner and Hanhart. Among the disorders in which elevated concentrations of tyrosine have been reported, this disorder appears to be a true hypertyrosinemia or tyrosine intoxication in the sense that the clinical manifestations are a consequence of the elevated levels of tyrosine. It has been referred to as "tyrosinemia type II".

The enzymatic site of the defect in oculocutaneous tyrosinemia is in the hepatic tyrosine aminotransferase (TAT, L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5) (Figure 21.1). There are two separate tyrosine aminotransferases, one in the cytosol and the other in the mitochondria. In this disorder, it is the cytosolic enzyme that is deficient [11–15]. The activity of the mitochondrial enzyme is normal. The gene for TAT is located on chromosome 16 at q22.1-22.3 [16, 17]. The gene has been cloned and sequenced [18]. A number of mutations have been defined [17, 18].

CLINICAL ABNORMALITIES

The most important manifestations of oculocutaneous tyrosinemia are those involving the eye [1, 2, 19], because they can lead to scarring of the cornea and permanent visual impairment. Ocular manifestations, such as lacrimation, photophobia, pain in the eye, or a history of red eyes are usually the initial manifestations of the disease and continue to be the most regularly encountered (Figure 21.2). Ocular symptoms may begin as early as the first day of life [20] and usually within the first years, but onset can be as late as 38 years [21], and documented patients have been asymptomatic [21]. Many patients have had symptoms of the eyes without cutaneous manifestations [5, 22–24], but others [6, 8, 24] had the reverse situation. Corneal ulcers are dendritic (Figures 21.3 and 21.4). The keratitis may resemble the dendritic keratitis of herpes.

The ocular and the cutaneous lesions in this disease are the result of the accumulation of tyrosine. Intense burning of the eyes, hands, and feet have been observed within an hour of the administration of 0.7–0.8 mmol of tyrosine per

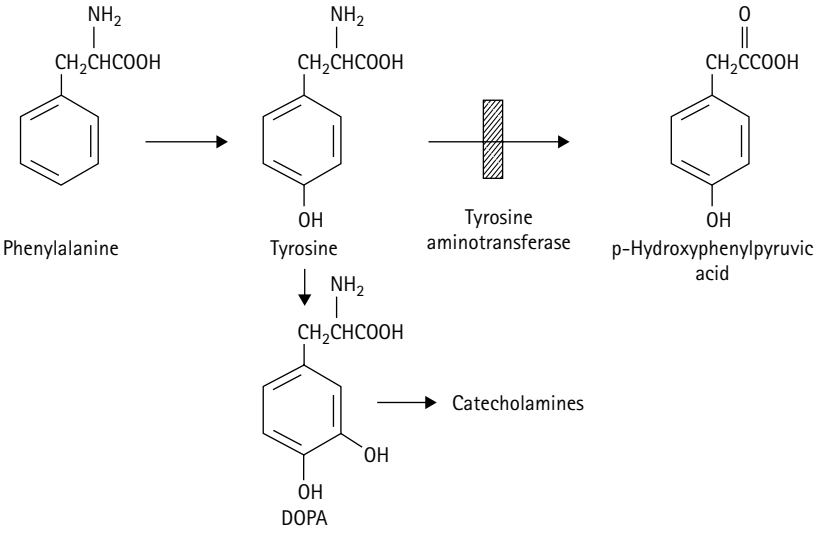

Figure 21.1 Metabolic pathways for tyrosine and the site of the defect in oculocutaneous tyrosinemia at the tyrosine aminotransferase step.

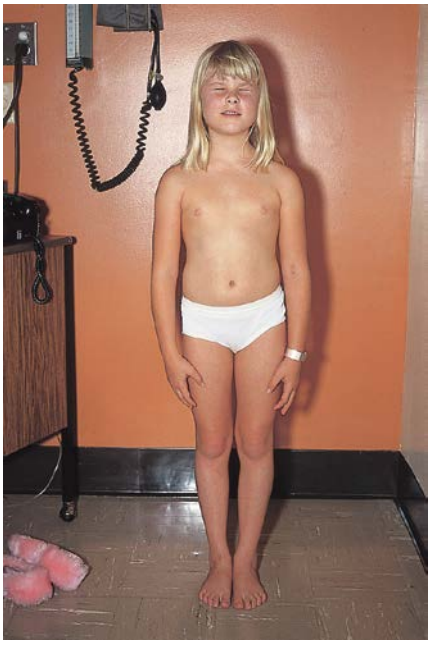

Figure 21.2 KP: A nine-year-old girl with oculocutaneous tyrosinemia [2]. She had recurrent photophobia and conjunctival reddening from at least six months of age. Development was mildly impaired.

kilogram [25], and erythema and pain have been observed in cutaneous lesions after a load of tyrosine [26]. Rats given a diet high in tyrosine develop keratitic ocular lesions [27]. Ocular abnormalities have been observed in other disorders in which tyrosine accumulates [28, 29].

The cutaneous lesions are painful keratoses which occur particularly on the peripheral pressure-bearing areas of the palms and soles (Figures 21.5–21.7) [30, 31]. They may occur near the tips of the digits. Subungual lesions may be found. Typical hyperkeratotic lesions are papular, well-demarcated plaques with irregular borders. Diameters up to 2 cm are common, but lesions may be larger and may be hollow or eroded, progressing to crusted, hyperkeratotic

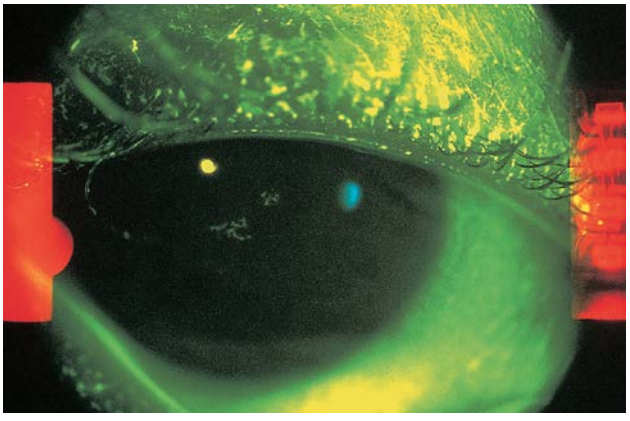

Figure 21.3 Corneal lesions of KP at nine years of age. The small dendritic lesion stained weakly with fluorescein. The lesion was slightly elevated. There was no ocular inflammation at the time. (Photograph kindly provided by Dr Perry Binder, San Diego, CA, USA.)

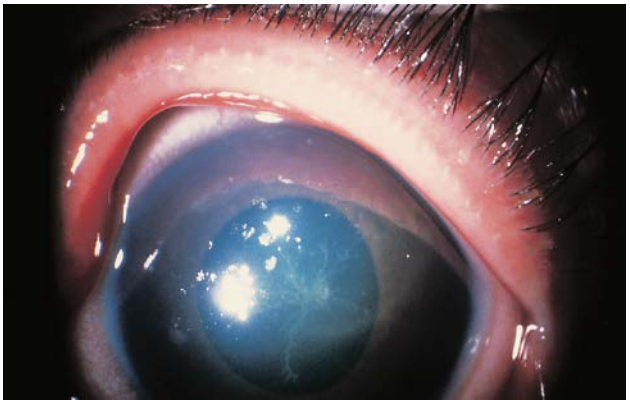

Figure 21.4 Corneal lesions in JF, a three-month-old patient with oculocutaneous tyrosinemia. The appearance was dendritic, but the lesions were elevated, opaque, and had mucoid material on the surface. The underlying dendritic figure stained weakly with fluorescein. (Illustration kindly provided by the US Naval Hospital, San Diego, CA, USA.)

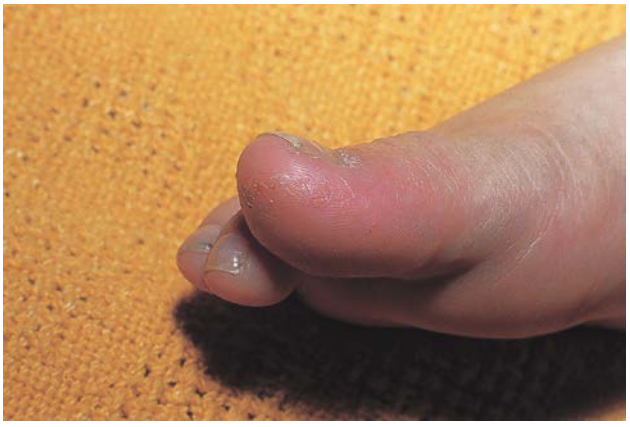

Figure 21.5 The lesion on the left great toe of KP was thickened, scaly, and cracked. Plasma concentration of tyrosine was 912 mmol/L (16.5 mg/dL).

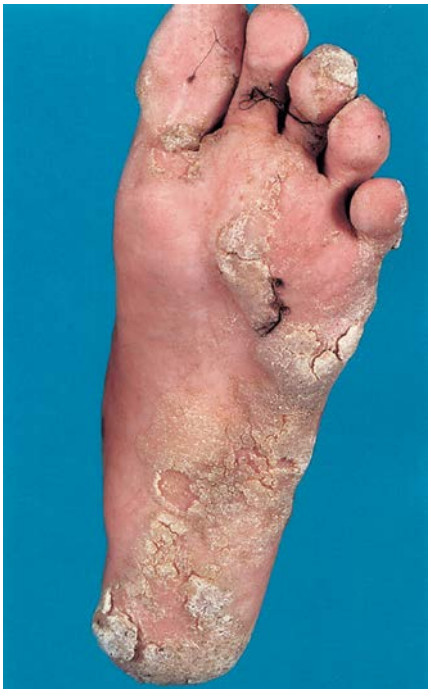

Figure 21.6 The foot of a Saudi patient with oculocutaneous tyrosinemia [34] who presented with these painful hyperkeratotic placques.

areas. They are not pruritic. They are painful and may be associated with hyperhydrosis [32]. They may be heralded by the appearance of blisters. Pain may be so severe that the patient will not walk. Skin lesions may be seen early in life or as late as the second decade [24]. Grayish hyperkeratotic plaques may be seen on the knees, elbows, or ankles. Hyperkeratoses have also been reported on the tongue [33]. Skin biopsy may reveal acanthosis and parakeratosis, as well as hyperkeratosis [30], none of them very specific findings. Electron microscopy may reveal unique thickening of the granular layer and increased tonofibrils and keratohyalin in the palmar and plantar skin along with large numbers

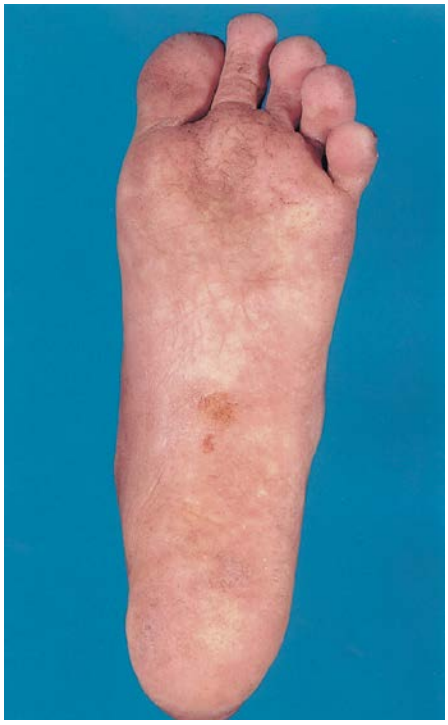

Figure 21.7 The foot of the same patient six weeks after dietary restriction lowered blood concentrations of tyrosine.

of microtubules and unusually tight packing [34]. Crystals of tyrosine were not seen, but they have been seen in the cornea.

Neurologic features of the disease are quite variable [2, 24]. About half of the patients reported have had impaired mental development, a few severely so [11, 26, 35], but in most of them the level of intelligence was not very low [6, 8, 30, 33, 36]. Some have been described as having low–normal intelligence [8, 27, 28, 37, 38]. Two patients had normal intelligence, but had a learning disability [2, 35]. Among the patients described, there was no obvious relationship between the levels of intelligence and the levels of tyrosine in the blood [2], and it is not clear whether impairment of mental development is an integral component of the syndrome or a reflection of ascertainment bias and the frequency with which such individuals are studied for the possible occurrence of metabolic disease. Magnetic resonance imaging (MRI) of the brain has been reported to reveal demyelination of white matter [39].

Hyperactivity has been observed in a number of patients, as well as abnormal language development [19]. An infant may be irritable. The first patient described [1] had self-injurious behavior, but had severely impaired mental development; this type of behavior has not been seen in others. Convulsions and microcephaly have been reported [40].

Maternal tyrosinemia has been documented to have adverse effects on the fetus [41]. An untreated 28-year-old woman whose disease was untreated, had two pregnancies with tyrosine levels at around 1302 μmol/L. Both infants had microcephaly and delayed development. One had maxillary

hypoplasia. Two other infants of untreated women had microphthalmia and impaired mental development [40].

GENETICS AND PATHOGENESIS

Transmission of oculocutaneous tyrosinemia is autosomal recessive. Consanguinity has been documented [4, 6, 30, 35, 36], as has the occurrence of more than one involved sibling with normal parents [8, 19, 36]. The disease has been seen in a wide ethnic and geographic distribution, but more commonly in Italians. A registry has been developed in Italy [40]. It is also prominent in Arab populations [42].

Heterozygote detection and prenatal diagnosis have not been reported, but this should be possible in those families in which the mutation is known.

A fluorometric procedure developed permitted the initiation of programs of neonatal screening for elevated concentrations of tyrosine [43]. These have been supplanted by the tandem mass spectrometry (MS/MS) programs of expanded neonatal screening.

An animal model for oculocutaneous tyrosinemia is available in mink, where it produces a disorder known as "pseudodistemper" in which there are exudative lesions of the eyes and volar skin [44]. The activity of tyrosine aminotransferase is reduced, and there is a reduced amount of hepatic cytosolic immunoreactive protein in these animals. There is also a canine model in German Shepherds [45].

Tyrosine aminotransferase normally converts tyrosine to p-hydroxyphenylpyruvic acid (see Figure 21.1). It is the rate-limiting step in the metabolism of tyrosine. The enzyme is highly regulated. Transcription is induced by glucocorticoids and cyclic AMP [46]. It is also developmentally regulated and human neonatal levels of activity are low [47]. The enzyme is a dimer that is phosphorylated and acetylated at its N-terminus. Pyridoxal phosphate is bound to lysine in the enzyme protein [48]. The activity of the enzyme has been measured in the liver of patients [1, 15, 29, 48–50] and found to be low.

The gene for TAT has been sequenced in human [18], mouse [51], and rat [52]. It contains 12 exons spanning 10.9 kb. The mRNA is 2.75 kb. The 50.4 kDa protein has 454 amino acids. A rearrangement in the structural gene of one patient was demonstrated by Southern blot analysis [17]. Among a number of point mutations reported [53, 54], an R57X is frequent in Italian patients. Two missense mutations, G362V and R4331Y, converted a glycine to a valine and an arginine to an asparagine. There were two splice-site mutations and three conversions to stop codons. Two novel mutations, C151Y and L273P in exons 5 and 6 were found in two consanguineous Tunisian families [42]. To date, it has not been possible to correlate phenotype with genotype.

When the activity of the enzyme is deficient, tyrosine accumulates, and tyrosine is the only amino acid that accumulates. Reported levels in the blood have generally ranged between 1100 and 3300 μmol/L (20 and 50 mg/dL) (Table 21.1) [1, 12, 21, 24, 40, 55]. A level of 1000 μmol/L appears to be a threshold level below which symptoms do not occur [2, 31]. Younger patients may have higher levels, and this is consistent with higher intakes of protein. We have observed higher levels during winter than in summer and could correlate this with a decrease in protein consumption in summer, even in San Diego, and despite no change in the diet prescribed, or in symptoms [2]. This seasonality has also been observed in Tunisians [42].

Table 21.1 Oculocutaneous tyrosinemia

	Plasma tyrosine concentrations (μmol/L)
Untreated patients on diagnosis	1100–2800
KP: ad lib diet – no sx	1215
KP: max during 11 months with no symptoms	1099
JF: asymptomatic	1073
Normal newborn	25–103
Child/adult	30–90

Prior to diagnosis, the mother had noted that symptoms regularly improved during the summer. Some patients have avoided protein-containing foods [19].

The tyrosinemia in this disorder is generally considerably greater than in other forms of tyrosinemia. Furthermore, analysis of the amino acids of the blood permits its distinction from transient tyrosinemia of the newborn because concentrations of other amino acids, particularly phenylalanine and methionine, are not elevated. It may be distinguished from hepatorenal tyrosinemia by the absence of a generalized aminoaciduria.

In oculocutaneous tyrosinemia, the excretion of tyrosine in the urine is increased to 180 and 2000 mmol/mol creatinine [4, 13, 56]. Acetyltyrosine is also found in the urine [13] when serum concentrations of tyrosine exceed 2500 μmol/L. Tyrosine may be converted to tyramine, and this compound may be found in the urine [13, 14]. Elevated concentrations of tyrosine are also found in the cerebrospinal fluid (CSF). Levels of 190–450 μmol/L have been reported [57, 30, 33].

Analysis of the organic acids of the urine reveals large amounts of p-hydroxyphenylpyruvic acid, p-hydroxyphenyllactic acid, and p-hydroxyphenylacetic acid. The excretion of large amounts of p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid in the urine seems at first to be inconsistent with the site of the metabolic block. It is explained (Figure 21.8) by the widespread distribution of the other transaminase, mitochondrial tyrosine aminotransferase (aspartate aminotransferase, EC 2.6.1.1), in tissues other than liver, which lack the hydroxylase that catalyzes the conversion of p-hydroxyphenylpyruvic acid to homogentisic acid [58]. Accumulated tyrosine found in

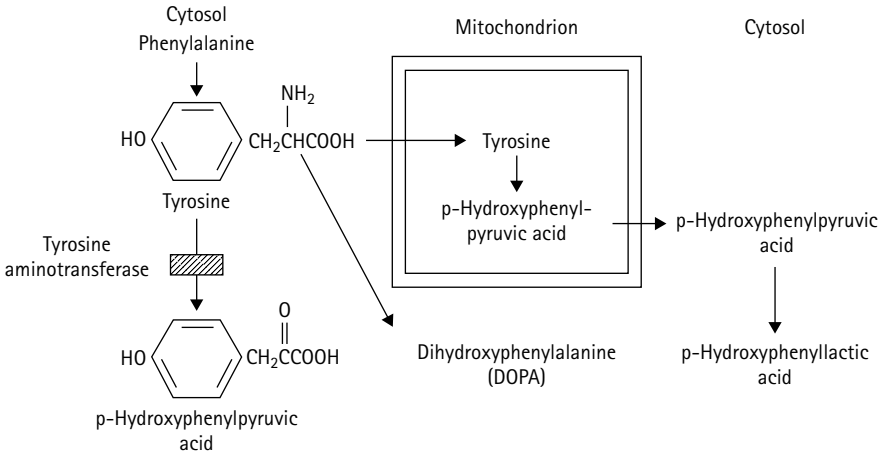

Figure 21.8 Metabolic interrelations in deficiency of hepatic cytosolic tyrosine aminotransferase. The site of the defect is on the left. The mechanism for the excretion of p-hydroxyphenylpyruvic acid is illustrated. Accumulated tyrosine becomes a substrate for the mitochondrial tyrosine aminotransferase which leads to the formation of p-hydroxyphenylpyruvic acid. In the liver, this compound is readily converted to homogentisic acid and further oxidized, but this enzyme is widely distributed among other tissues of the body, and therefore p-hydroxyphenylpyruvic acid accumulates and is excreted in the urine. (Reprinted with permission from Nyhan WL. *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton-Century-Crofts, 1984.)

the blood is converted to p-hydroxyphenylpyruvic acid in tissues such as muscle. This compound is readily reduced to p-hydroxyphenyllactic acid [59]. Both p-hydroxyl compounds are then transported in the blood to the kidney, where they are effectively cleared and excreted in the urine [60].

TREATMENT

The treatment of oculocutaneous tyrosinemia consists of the institution of a diet low in tyrosine and phenylalanine. This effectively lowers concentrations of tyrosine in body fluids. Clinical symptomatology promptly resolves. Preparations (Tyrex (Ross) and Xphe XtyrAnalog, Maxamaid (SHS)) are available which are low in tyrosine and phenylalanine and simplify the preparation of formulas for the feeding of infants with tyrosinemia. Attention to compliance is important because treatment can prevent permanent ocular damage. The fact that symptoms of the disorder may be quite uncomfortable assists in the compliance of older patients. Whether early therapy prevents impaired mental development is not clear, but early dietary management is prudent. There is excellent correlation between the concentration of tyrosine in the plasma and the intake of the amino acid and its precursor (Figure 21.9). Reasonable levels of control and an absence of symptoms are readily achieved using acceptable diets in childhood, as well as in infancy.

Optimal blood levels have not been defined, but most patients are free of symptoms as long as the plasma concentration of tyrosine is below 550–700 μmol/L [2, 61–63]. Treatment with oral etretinate has been reported to improve skin lesions without changing levels of tyrosine [32], but this seems less than desirable because the skin is usually easier to control than the eye. Occasional

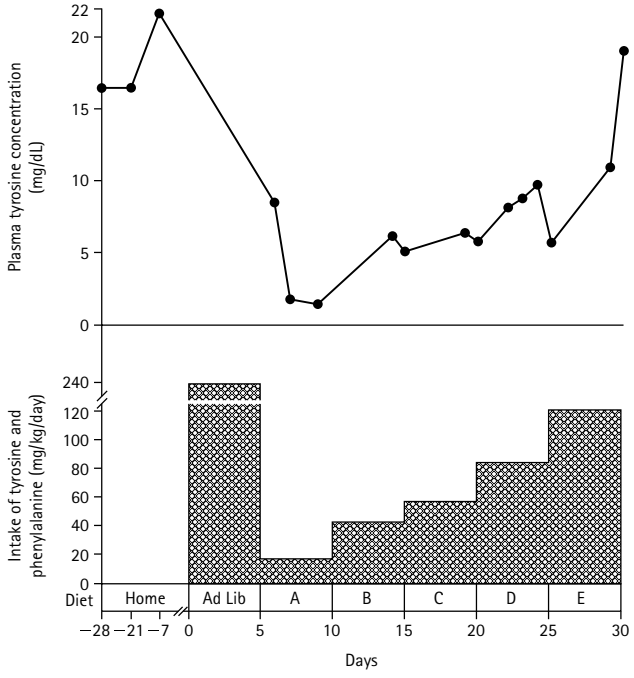

Figure 21.9 Relation of the plasma concentration of tyrosine to the intake of tyrosine and phenylalanine. (Reprinted with permission from Nyhan WL. *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton-Century-Crofts, 1984.)

noncompliance is not a problem as long as control is maintained most of the time.

Experience with maternal tyrosinemia indicates that dietary control during pregnancy would be prudent [41]. On the other hand, a number of normal offspring of mothers with this disease have been recorded [21, 39], so control of tyrosine levels can be rewarding.

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22

Hepatorenal tyrosinemia/fumarylacetoacetate hydrolase deficiency

MAJOR PHENOTYPIC EXPRESSION

Hepatocellular degeneration leading to acute hepatic failure, or chronic cirrhosis and hepatocellular carcinoma; renal Fanconi syndrome; peripheral neuropathy; hypertyrosinemia; succinylacetonuria; and deficiency of fumarylacetoacetate hydrolase.

INTRODUCTION

Hepatorenal tyrosinemia, which has been referred to as tyrosinemia type 1, tyrosinosis, or hereditary tyrosinemia, was first reported by Sakai and Kitagawa in 1957 [1–3]. The patient reported was the product of a consanguineous mating, who developed progressive liver disease, which led to death following hematemesis and hepatic coma at three years of age. In addition, the patient had rickets, which was resistant to vitamin D. The major metabolic products in the urine were p-hydroxyphenyllactic acid, p-hydroxyphenylpyruvic acid, and p-hydroxyphenylacetic acid, as well as tyrosine. Gentz and colleagues [4], in a report of seven patients with the disease, first characterized the renal component as Fanconi syndrome. It was noted that patients had neurologic crises reminiscent of porphyria [5, 6], and this led to the recognition that δ-aminolevulinic acid was excreted in large amounts [6–9]. Lindblad and colleagues [10] reported that succinylacetone, which they found in the urine of these patients, is an inhibitor of the synthesis of porphobilinogen from δ-aminolevulinic acid. They reasoned that the fundamental defect was in the activity of fumarylacetoacetate hydrolase (Figure 22.1). This was confirmed enzymatically by these investigators [11] and others [12–14].

The gene has been cloned [15, 16] and mapped to chromosome 15q23-25. Mutations have been identified [17], including founder mutations in French-Canadian Quebec and in Finland, both places where the disease is prevalent. The Quebec mutation is a splice mutation IVS12+5G-A [18], and that in Finland is W262X [19]. The discovery of a therapeutic agent 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) (nitisinone) represents a major advance in the management of this disease [20, 21].

CLINICAL ABNORMALITIES

The clinical course of hepatorenal tyrosinemia has generally followed one of two patterns: an acute or a chronic form. The former has sometimes been referred to as the French-Canadian type and the latter the Scandinavian type, but of course there is considerable overlap [4, 5, 14, 22]. Most patients have had acute presentations. Symptoms develop in early infancy, and they are those of acute hepatic decompensation. Hepatic failure and death occur usually under one year of age. However, some infants with an acute onset of hepatic disease survive to go on to display a chronic disease just like those patients with the chronic form. The differential diagnosis of hepatic failure is given in the Appendix. Until recently, most of these children died at younger than ten years of age. Only one patient of those described early survived to the age of 20 years [22]. The year one mortality for those presenting with symptoms by two months was 60 percent; of those presenting between two and six months, it was 20 percent; and of those presenting after six months it was 4 percent [23]. Prognosis has changed dramatically with currently available therapy.

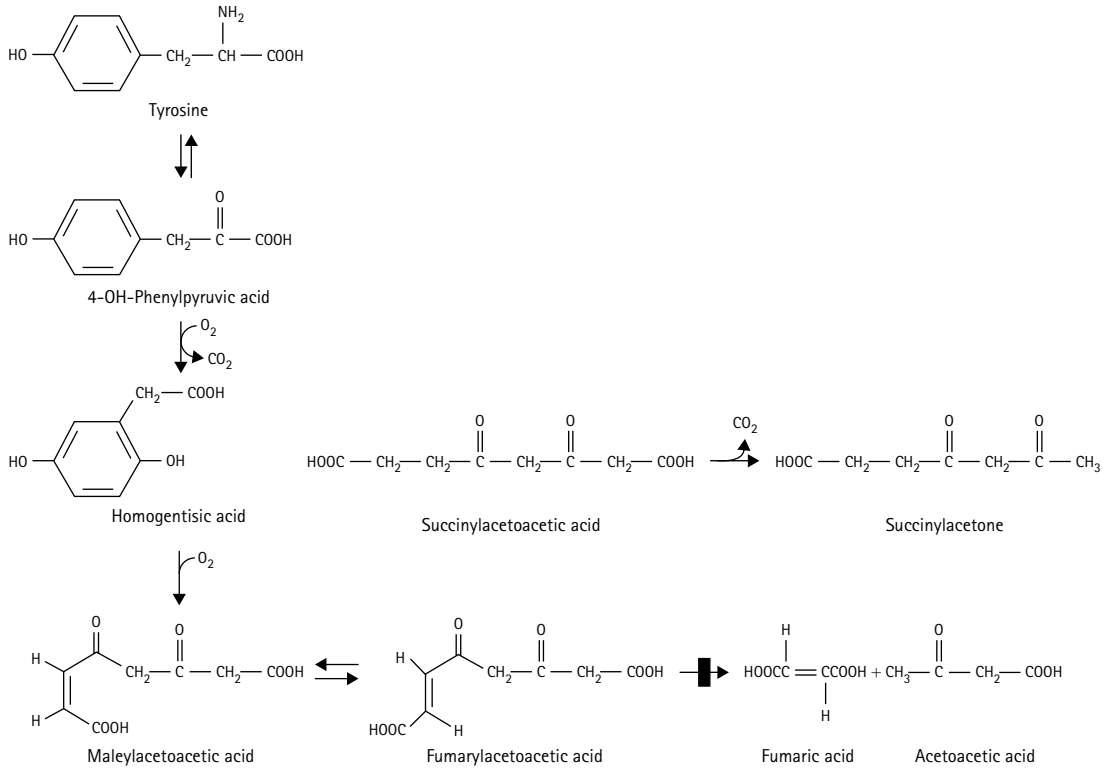

Figure 22.1 Metabolism of tyrosine and phenylalanine. The site of defect hepatorenal tyrosinemia is in fumarylacetoacetate hydrolase.

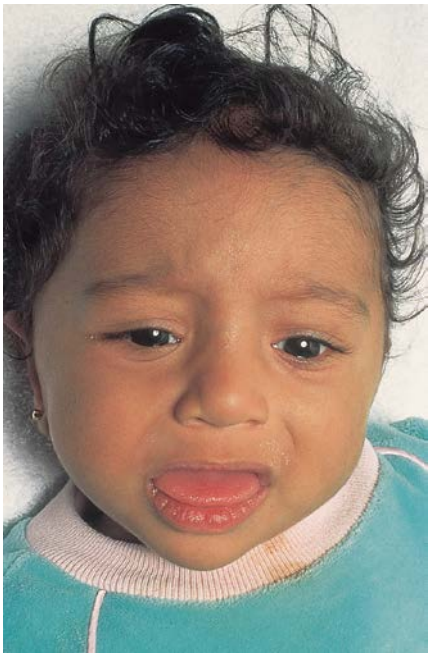

Figure 22.2 AY: A seven-year-old girl with hepatorenal tyrosinemia.

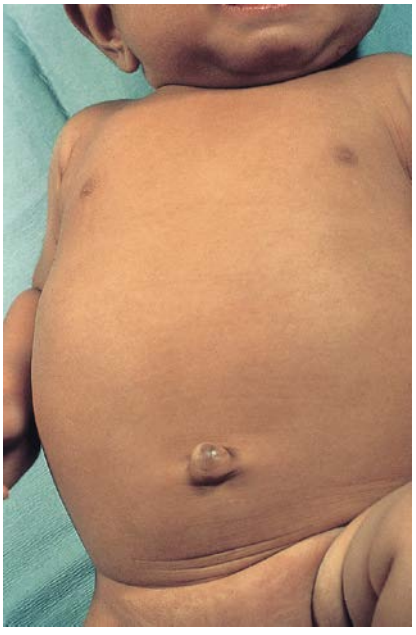

Figure 22.3 AMQ: A boy with hepatorenal tyrosinemia. The abdominal enlargement resulted from the liver which was palpable 4 cm below the costal margin.

The earliest and the major effect of the disease is on the liver. Abdominal distension and failure to thrive are prominent, and may be associated with vomiting and/or diarrhea (Figures 22.2–22.4). The acute hepatic crisis is the most common early presentation. The infant may appear acutely or desperately ill and have jaundice and ascites along with hepatomegaly [24–26]. There may be gastrointestinal bleeding. Hypoglycemia may be a presenting symptom. Hepatic crises may be triggered by infection.

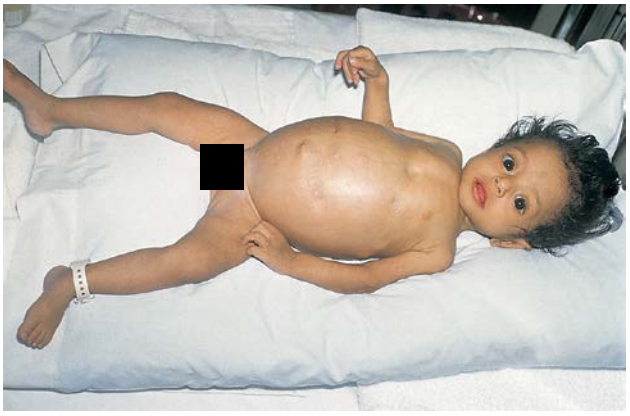

Figure 22.4 JQ: A girl with hepatorenal tyrosinemia. She had cirrhosis with abdominal enlargement and ascites.

Several infants have been noted by their mothers to have a peculiar sweet odor. A boiled cabbage-like odor in some patients has been related to a metabolite of methionine, 2-oxo-4-methiolbutyric acid [27–30].

Transaminase levels in the blood may be normal or slightly elevated. The rare elevation over 1000 IU/L indicates substantial damage to hepatic cells. α-fetoprotein may be markedly elevated, ranging from 100,000 to 400,000 ng/mL. Coagulation factors may be abnormal, and there may be bleeding. Prothrombin time (PT) and partial prothrombin time (PTT) may be markedly prolonged. Coagulopathy is characteristically unresponsive to vitamin K. Jaundice is uncommon early in this disease.

One of our patients presented with bleeding and was investigated as a problem in coagulation before chemical evidence of hepatic disease was identified. Patients may present with epistaxis or intestinal bleeding [31]. Elevated levels of PT and PTT may be found even in asymptomatic infants discovered by newborn screening. An infant presenting with liver disease and hypoglycemia may be thought to have Reye syndrome. Between acute crises the liver is enlarged.

The chronic liver disease picture is that of hepatic cirrhosis. The differential diagnosis of hepatic cirrhosis in infancy is given in the Appendix. The pathologic picture is that of macronodular cirrhosis [32]. Splenomegaly develops. There may be acute crises of increased hepatocellular damage, often precipitated by infection, and these may lead to hepatic failure. Esophageal varices may develop, and they may be complicated by bleeding.

A more common complication is the development of hepatocellular carcinoma [33, 34]. The risk of this complication has been variously reported. In a series of 42 patients reported in 1976 [35] from the United States, 37 percent of those over two years of age developed carcinoma, while information from an international series yielded an incidence of 18 percent of those over two years [36]. Detection of nodules by computed tomography (CT) scan or ultrasound appears to be quite reliable, because histologic examination of 18 livers from patients subjected to liver transplantation failed to reveal focal carcinomas in patients not found to have nodules by those modalities [37]. CT should be performed with and without contrast. Liver cancer has been documented as early as 33 months of age [38]. Even younger, a 15-month-old patient was found to have a carcinoma following a presentation at five months with acute hepatic failure and a good response to NTBC with a ten-fold drop in α-fetoprotein, which then rose to 100,000 ng/mL [39]. A significant rise in the level of α-fetoprotein may herald the onset of carcinoma, but carcinoma was found in a patient whose level was only 87 ng/mL [3]. Patients should be monitored regularly by CT, magnetic resonance imaging (MRI), or ultrasound, and nodules should be biopsied.

Renal disease is another characteristic feature of this disease. Among 32 patients [40], 47 percent had enlargement of the kidneys, often palpable [31]; 47 percent had increased echogenicity of the kidneys and 16 percent had nephrocalcinosis. In another eight patients [41], 50 percent had nephromegaly. The renal tubular disease is that of a typical renal Fanconi syndrome in which there is phosphaturia, aminoaciduria, and often glycosuria. There may be proteinuria. Systemic metabolic acidosis may result from renal tubular dysfunction. The phosphate losses lead to hypophosphatemia and clinical rickets (Figures 22.5–22.7). There may also be a variable reduction in

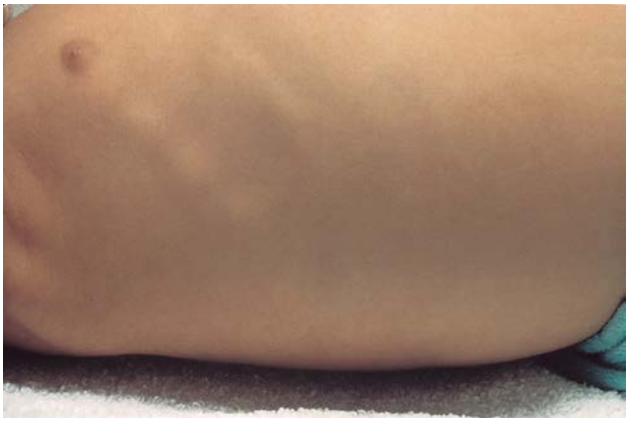

Figure 22.5 AY: Illustrating the rachitic rosary.

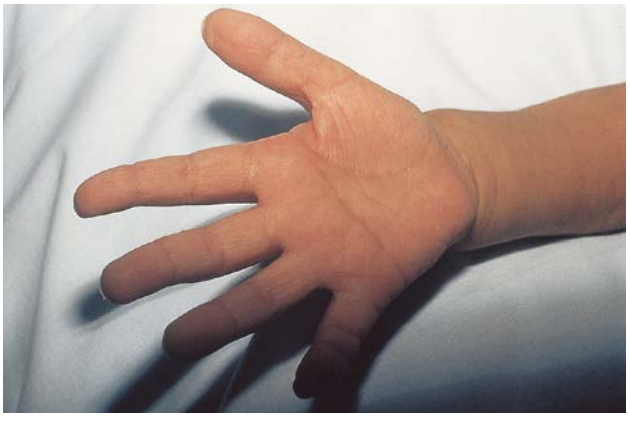

Figure 22.6 AY: The wrist was enlarged because of rachitic changes at the ends of bones.

Figure 22.7 AMQ: The wrist was also enlarged.

glomerular function. In the series of 32 patients [40], 48 percent had decreased glomerular filtration; 82 percent had aminoaciduria, 67 percent hypercalcinuria, and 59 percent renal tubular acidosis. Affected infants have been observed to have vitamin D-resistant rickets at less than four months [25], which is unusual.

Neurologic crises of pain and paresthesia are a result of peripheral neuropathy [42–45]. These may occur in as many as 42 percent of patients. Crises may be mistaken for porphyria [43]. There may be extensor hypotonus or the patient may have hypertonia. Systemic, autonomic signs include hypertension, tachycardia, and ileus. Pain usually begins in the legs. The patient may position the head and trunk in extreme hyperextension and may be thought to have opisthotonus or meningismus [29]. Muscular weakness may progress to paralysis requiring artificial ventilation [42]. Self-injurious behavior has been observed. Some patients have had seizures [43], some of them associated with hyponatremia [8]. Death may occur during a neurologic crisis [44, 45]. During most crises, consciousness is normal. These crises are not associated with hepatic relapse. Most crises subside in 1–7 days and resolve slowly, but there may be residual weakness. Intelligence is usually normal.

Three infants have had obstructive hypertrophic cardiomyopathy [46, 47], and this may be fatal [46]. Two patients have had macroglossia [7, 36], and there may be macrosomia. Pancreatic islet hypertrophy is common, but usually asymptomatic. Hypoglycemia can usually be attributed to hepatic disease.

GENETICS AND PATHOGENESIS

Hepatorenal tyrosinemia is transmitted in an autosomal recessive fashion [26, 48]. Consanguinity has been documented in a number of families [49]. A particularly high frequency of 1.46 per 1000 births has been recorded in a French-Canadian isolate in the Chicoutimi–Lac St Jean region of northeastern Quebec [49, 50], where the carrier rate is one in 20 [47]. An overall incidence of 0.8 per 10,000 births was observed in the French-Canadian population of Quebec. The prevalence has approximated at 1 in 100,000 [51] from newborn screening programs in Scandinavia. Founder effects have been elucidated in the French-Canadian population [52, 53]. The disease is frequent in French-Canada and relatively so in Scandinavia, but it may be found in any geographic or ethnic background.

The molecular defect in hepatorenal tyrosinemia is in the hepatic fumarylacetoacetic acid hydrolase (fumarylacetoacetase, EC 3.7.1.2) (see Figure 22.1). This was originally proposed on the basis of the accumulation of succinylacetone [10]. Deficiency of this enzyme was then documented by assay of activity in liver [12]. The level was 6 percent of normal in six patients with the acute disease and 20 percent in two patients with the chronic form. The activity of maleylacetoacetic acid hydrolase was also deficient in some samples of liver. A problem with enzyme assay is that in the presence of liver disease, the activities of many enzymes are reduced, but the fundamental enzyme deficiency may also be demonstrated in lymphocytes and fibroblasts [54]. The gold standard in the diagnosis of this disease is the demonstration of succinylacetone in the urine.

Heterozygote detection has been carried out by the assay of fumarylacetoacetate hydrolase activity in fibroblasts and lymphocytes [55]. Obligatory heterozygotes have had a mean level that is 50 percent of normal, but considerable variation and the possibility of pseudoalleles make this unreliable. Where the mutation is known, or in populations like that of Quebec where a small number of mutations is responsible, molecular testing is the preferred method for the detection of carriers.

Prenatal diagnosis has been accomplished by assay of the enzyme in cultured amniocytes or chorionic villus material [55–57]. It has also been accomplished by the direct assay of concentrations of succinylacetone in amniotic fluid [58, 59], and this is thought to be the method of choice. However, at least one affected infant has been missed in this assay [60]. In families in which the mutation is known, molecular methods are ideal.

The gene has been localized to chromosome 15q23-25 [15]. The gene contains 14 exons over a span of 30–34 kb [61]. A number of restriction fragment length polymorphisms (RFLP) have been identified, and these RFLPs may be used for carrier detection and prenatal diagnosis [62]. Ten haplotypes were found with five RFLPs in the French-Canadian population. Haplotype 6 was strongly associated with the disease; its frequency was 90 percent in French-Canadian and 96 percent in Saguenay–Lac-Saint-Jean. A considerable number and variety of mutations have been identified (Table 22.1), and heterogeneity has been identified, even in the French-Canadian population [61, 63]. In a French-Canadian patient, an A-to-T transversion changed an asparagine to isoleucine at position 16 (Figure 22.8) [17]. The IVS12+5G-A mutation is more common [18]. In a Norwegian patient, a missense mutation changed alanine at 134 to aspartic acid [61]. A splice-site mutation resulting

Table 22.1 Variants of fumarylacetoacetate hydrolase in hepatorenal tyrosinemia

Type	mRNA	CRM	Enzyme activity
A	11	0	0
B	1	1	1
C	11	11	0
D	0	0	0

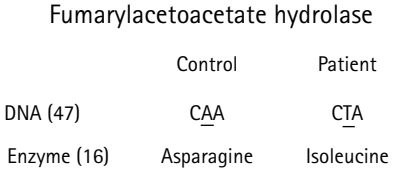

Figure 22.8 Variant fumarylacetoacetate hydrolase gene and enzyme in a French-Canadian patient with hepatorenal tyrosinemia. The numbers in parentheses indicate nucleotide 47 in the gene and amino acid 16 in the protein.

from a G-to-A transition was found to lead to deletion of exon 12 in a French-Canadian patient [64]. Among 62 patients of varied ethnicity the IVS12+5G-A+5, the common French-Canadian mutation, was the most common found in 32 alleles from the United States, Europe, Pakistan, and Turkey [19]. A Scandinavian mutation c.1009G>A was a splice mutation, as was c.192G>T found in Pakistanis. Splice-site mutations were also common in 92.8 percent of alleles among 29 patients from the Mediterranean area [65]; IVS6-1G-T was the most common.

In a recent assessment [66], 95 total mutations have been reported worldwide, including 45 missenses mutations, 23 splice defects, and 13 nonsense mutations. A relatively high frequency of mutations, one in 74,800, were found in Norway [65], where three mutations were novel, c.615delT, c.744delG and c.835delC, all leading to frameshift and premature termination.

The mutation found most frequently worldwide was c.1062+5G>A, IVS12+5G>A.

A pseudodeficiency allele p.R341W has been found in normal individuals with low activity of the hydrolase enzyme [67]. Mutations have been shown to produce mRNA without enzyme activity or cross-reacting material (CRM); mRNA and CRM without enzyme activity; mRNA, CRM, and some activity; as well as no mRNA. Patients with early onset hepatic failure tend to be CRM-negative.

The deficient enzyme is on the catabolic pathway for tyrosine, and this is the cause of the hypertyrosinemia (see Figure 22.1). Fumarylacetoacetate accumulates and is converted to succinylacetoacetate and to succinylacetone. In hepatorenal tyrosinemia, concentrations of tyrosine usually range from 170 to 660 μmol/L (3–12 mg/dL).

Increased quantities are also excreted in the urine. Of the tyrosyl compounds found in the urine, p-hydroxyphenyllactic acid is the most prominent; p-hydroxyphenylpyruvic acid and p-hydroxyphenylacetic acid are also present in appreciable quantities. Patients often have elevated concentrations of methionine in the blood. Hypoglycemia is common, especially in the acute illness. In chronic cirrhosis or after treatment, tyrosine concentrations may be normal. On the other hand, during the acute stages of hepatocellular damage many other amino acids may be found in elevated amounts in the serum, including cystathionine, proline and hydroxyproline. These patterns, along with the tyrosine, are reflected in the urinary excretion of amino acids. They are superimposed on the generalized aminoaciduria that results from the renal tubular aspects of the disease. Patients also have phosphaturia and hypophosphatemia. The presence of reducing substance completes the picture of the renal Fanconi syndrome. The sugar is usually glucose, but other sugars have been reported [4, 68]. With progression, there is systemic acidosis, increased potassium loss, and hypokalemia.

The urinary excretion of δ-aminolevulinic acid is increased [8, 10, 69, 70]. Succinylacetoacetic acid and succinylacetone are found in the serum and the urine [10, 68], the direct consequence of the defective activity of fumarylacetoacetic acid hydrolase. Accumulated fumarylacetoacetic acid is reduced to succinylacetoacetic acid and decarboxylated to form succinylacetone. Succinylacetone has immunosuppressive activity [69]. It is also a powerful inhibitor of δ-aminolevulinic acid dehydratase [71], accounting for the increased excretion of δ-aminolevulinic acid and inhibition of the synthesis of porphobilinogen from δ-aminolevulinic acid. Succinylacetone can be found in spots of blood dried on filter paper. Screening for hepatorenal tyrosinemia has been undertaken in a number of states and countries. It has recently been incorporated into expanded tandem mass spectrometry (MS/MS) programs with tyrosine as the key analyte. This has the problem that transient neonatal tyrosinemia triggers a positive screen, and the numbers are such that many programs have set the screen level so high that most patients with hepatorenal tyrosinemia would be missed. In addition, some patients with this disease have normal levels of tyrosine. Quebec now screens for succinylacetone. A liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of succinylacetone in dried blood spots was developed for use in a two-tier screening method for this disease [72]. It could also be useful in monitoring treatment in patients. A combined assay for succinylacetone, amino acids, and acylcarnitine has since been developed [73] which required a separate extraction step not a single MS/MS.

The pattern of laboratory findings in this disease is virtually unique. A combination of hypoglycemia, coagulopathy, tyrosinemia, succinylacetone, and very high α-fetoprotein is diagnostic.

Fumarylacetoacetate is an inhibitor of methionine adenosyltransferase, and this would lead to

hypermethioninemia [74], but methionine levels also increase nonspecifically in hepatocellular disease. Renal tubular dysfunction is thought to result from maleylacetoacetic acid by analogy with maleic acid, which can produce an experimental Fanconi syndrome in animals [75]. 4-Fumarylacetoacetate and maleylacetoacetate react with sulfhydryl compounds, and deficiency of glutathione has been documented in this disease [76]. Maleylacetone and succinylacetone can form glutathione adducts [77]. The accumulated products are highly reactive and can form stable adducts through the formation of Schiff bases with proteins and amino acids such as lysine by the alkylation of thiols and amino groups, and this could be a mechanism for production of disease. The acute porphyria-like episodes of peripheral neuropathy in this disease are thought to result from the inhibition by succinylacetone of δ-aminolevulinic acid hydrolase and hence the formation of porphobilinogen.

An interesting phenomenon in this disease is the occurrence of revertant nodules in which hydrolase activity is normal [78, 79]. The enzyme protein is present in these nodules in which at least one allele has mutated to the normal sequence. This, of course, could lead to a finding of normal activity in biopsied liver. This reversal to the normal genotype involved the reversion of a mutant AT nucleotide pair to GC the normal, and it was found in three different disease-producing mutations including the common splice-site mutation. In another example of mosaicism of normal and mutant phenotypes in patient liver, a new mutation upstream of the primary mutation suppressed the abnormal splicing [80].

TREATMENT

Treatment of this disease has been revolutionized by the discovery of NTBC (Figure 22.9) [20, 81]. Restriction of the dietary intake of phenylalanine and tyrosine will lower concentrations of tyrosine, and improvement in renal tubular function has been reported [35, 82–84]. Coagulation problems are also responsive. However, hepatic disease may progress despite dietary treatment.

Acute hepatic dysfunction must be treated aggressively. Energy and nutrition may be provided parenterally, as well as the management of fluids and electrolytes. Intake of phenylalanine and tyrosine is stopped temporarily. In liver failure, transplantation of a liver is the only answer. In a neurologic crisis, attention to respiration and assistance when necessary are mandatory.

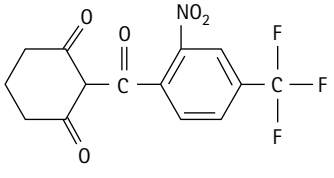

Figure 22.9 NTBC, 2(2-nitro-4-trifluoromethylbenzoyl)-1, 3-cyclohexanedione.

Transplantation has also become the treatment choice for hepatocellular carcinoma [85, 86]. In recent years, transplantation has been undertaken prior to the development of nodules in order to prevent carcinoma [87–89]. Survival rates at 36 months following transplantation of the liver for this disease have been as high as 87 percent [89]. Tyrosyl compounds in the urine decreased to normal, while succinylacetone decreased, but as far as normal in only one patient [90]. Presumably, this succinylacetone is made in the kidney. The management of a patient with multiple hypodense hepatic nodules has become complex because of a report that nodules might represent nodular cirrhosis and fatty change [91], and a report that with medical treatment such lesions disappeared [92].

Excretion of δ-aminolevulinic acid also decreased, but remained somewhat elevated. Renal tubular reabsorption of phosphate and bicarbonate may become normal within five days of transplantation; glycosuria and aminoaciduria correct within two weeks [93].

The advent of therapy with NTBC has changed the readiness with which hepatic transplantation is performed in this disorder. The indication now is hepatic cancer. NTBC is a potent inhibitor of p-hydroxyphenylpyruvate dioxygenase [94]. Treatment with 1 mg/kg of this compound has led regularly to improvement in hepatic and renal function, and no side effects have been observed. Concentrations of succinylacetone and α-fetoprotein have decreased, and hepatic morphology has improved. Excretion of δ-aminolevulinic acid has decreased to near normal, and erythrocyte porphobilinogen synthesis increased. This appears to eliminate the neurologic crises of the disease in those properly treated [95]. NTBC has been approved by the US Food and Drug Administration (FDA) for the treatment of hepatorenal tyrosinemia. As of 2003, 369 patients had been treated [96], and treatment was continuing on 293. Withdrawals were 76, of whom 26 had died; 21 had liver failure, of which 12 died; 25 had developed hepatocellular carcinoma, of whom seven had died; and 54 had been transplanted, of whom eight died. Prior to NTBC, survival curves in this disease indicated few long-term survivors. Now, approximately 90 percent of those diagnosed before two years of age are alive, some as long as 12 years. The figure for those diagnosed late approximates 60 percent surviving 6–12 years. There have been only three hepatic cancers in those treated before two years of age, and one of these was present at diagnosis, before treatment. Improvement has been reported when NTBC was given during a neurologic crisis [96].

The effects of NTBC on renal function were reported [98] in five patients diagnosed at ages between five and 53 months. All had rickets and a renal Fanconi syndrome with lowered plasma phosphate prior to treatment. The phosphate became normal within one week of initiation of therapy with 1 mg/kg of NTBC in most patients and within two weeks in all. Other markers of renal dysfunction improved over the two weeks of study.

Experience with NTBC in the early treatment of patients detected by newborn screening [97] was positive; there was no mortality and better neurodevelopment in the new affected sibling than in older affected siblings.

NTBC has been employed in a daily dose of 1 mg/kg in infants without disease, detected by newborn screening [99]. In those with hepatic failure, the recommended dose is 2 mg/kg, allowing the dose to fall with growth to 1 mg/kg before increasing it. A diet low in tyrosine and phenylalanine is also necessary to avoid pathologic elevations of tyrosine. Imaging (MRI or CT) for hepatic disease is important for management. Monitoring of levels of succinylacetone is important to ensure against lapses of compliance with medications. In Quebec, concentrations of NTB1 are measured. Levels above 50 μmol are preferred [99]. Normal babies have been delivered of mothers treated with NTBC through pregnancy [99].

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23

Nonketotic hyperglycinemia

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal neonatal illness, absent or poor mental development, convulsions, myoclonus, hiccups, hypotonia progressive to spasticity, abnormal electroencephalogram (EEG), hyperglycinemia, hyperglycinuria, elevated cerebrospinal fluid (CSF) to plasma glycine ratio, and defective activity of the glycine cleavage system.

INTRODUCTION

Nonketotic hyperglycinemia (glycine encephalopathy) is the second most common inborn error of amino acid metabolism after phenylketonuria. Large amounts of glycine accumulate in body fluids; the increase in CSF is disproportionally high, resulting in an elevated CSF to plasma glycine ratio; and there is no demonstrable accumulation of organic acids. A majority of patients has the classic phenotype in which life-threatening illness begins in the early days of life, and most patients die if not maintained by the use of mechanical ventilation. Survivors usually display little cognitive development and often have virtually continuous seizures. The disease was first described by Gerritsen and colleagues in 1965 [1]. It was called "nonketotic hyperglycinemia" [2, 3] to distinguish it from other disorders, such as propionic acidemia (Chapter 2), in which hyperglycinemia occurs. The high concentration of glycine in CSF and the ratio of its concentration to that of plasma, provide the usual method of diagnosis. Analysis of organic acids of the urine is useful to exclude organic acidemia. Enzyme analysis is not generally available; the enzyme is fully expressed only in the liver and brain.

The molecular defect is in the glycine cleavage system (EC 2.1.2.1.0) (Figure 23.1), which is a multienzyme complex with four protein components [4]. These have been labeled the P-protein (glycine decarboxylase, GLDC), T-protein (aminomethyl transferase, AMT), H-protein (the lipoic acid containing protein), and L-protein (a lipoamide dehydrogenase). The P-protein contains the active form of pyridoxal phosphate and decarboxylates glycine with

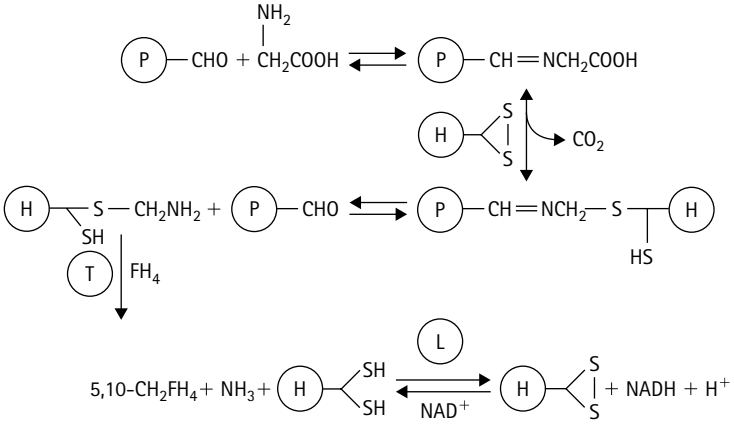

Figure 23.1 The glycine cleavage system. The protein components, circled, are labeled, P, H, T, and L. (Reproduced with permission from Nyhan WL. *The Metabolic Basis of Inherited Disease*, 5th ed. Stanbury JB, Wyngaarden JB, Fredrickson DS *et al.* (eds). New York: McGraw Hill, 1982: 564.)

release of CO_2. The aminomethyl group is then transferred onto the lipoic acid of the H-protein. The T-protein transfers the α-carbon as a methyl group from the H-protein onto tetrahydrofolate yielding methylene tetrahydrofolate with release of ammonia. Finally, the H-protein is reoxidized to the disulfide form by the L-protein.

In patients with nonketotic hyperglycinemia in whom the individual components have been studied, the majority has had defects in the P-protein. Defective activity of the T-protein has also been described, and variant nonketotic hyperglycinemia is caused by defects in the lipoylation of the H-protein or the biosynthesis of the iron sulfur clusters required for lipoate synthesis [5, 6]. The cDNA for the P- and T-proteins have been cloned and more than 200 mutations in the *GLDC* gene for the P-protein and more than 50 in the *AMT* gene have been defined [7–10].

CLINICAL ABNORMALITIES

In the classic phenotype, the infant appears normal at birth and there is a hiatus, usually up to 48 hours but ranging from a few hours to eight days, in which the patient remains well. Then, usually after the initiation of protein-containing feedings, lethargy develops, along with anorexia and failure to feed or later to suck. Feeding by nasogastric tube may be initiated. There may be some vomiting, but this is usually not a prominent feature. Lethargy is progressive to coma, and within 24–48 hours of the first symptom the patient is flaccid, completely unresponsive to stimuli, and apneic (Figure 23.2)

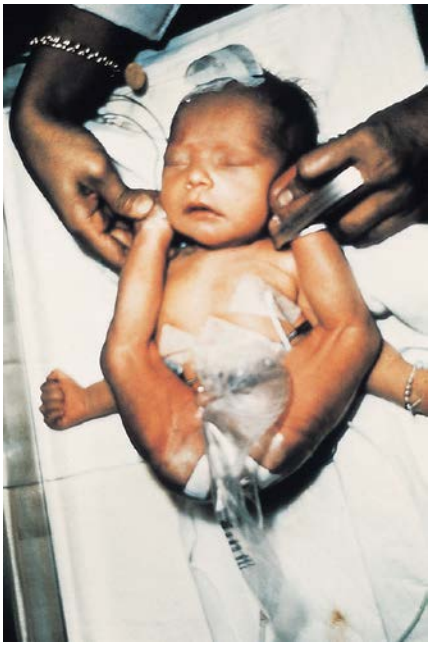

Figure 23.2 JS: A four-day-old patient with nonketotic hyperglycinemia. Following exchange transfusion, assisted ventilation could be discontinued, but the patient was still in the intensive care unit and unresponsive. Illustrated is the extreme hypotonicity.

[1–3, 11–18]. A majority of patients probably die at this point. Some are ventilated artificially using a respirator for long enough to permit the diagnosis. Subsequent treatment with exchange transfusion, peritoneal dialysis, or sodium benzoate may lead to the initiation of spontaneous respirations and the discontinuation of the respirator which also occurs spontaneously between 10 and 22 days of age. However, there is seldom much evidence of cerebral development and many patients die within the first year of life. The disorder is diagnosed in increasing fashion in neonatal intensive care units of major medical centers, but it is likely that as many or more die neonatal deaths without benefit of diagnosis.

In the infant, the cry may be high-pitched. Suck, grasp, and Moro responses are poor. Edema has been observed rarely [11, 17]. Seizures may be myoclonic or grand mal [15]. They are prominent in almost all patients and may be virtually continuous [1, 17, 19]. Hiccupping is common and often persistent [11] and, with some frequency, we have obtained historical evidence of recurrent prenatal hiccupping. Intermittent ophthalmoplegia or wandering eye movements have been described [20]. The EEG is usually diffusely abnormal [21–23]. The typical pattern of burst-suppression is one of periodic or pseudoperiodic areas of large-amplitude sharp waves on a low voltage background [21, 22]. The burst-suppression pattern has been observed as early as 30 minutes after birth [22]. This pattern, typical of the neonate, may change to hypsarrhythmia in later infancy. There may be multifocal epileptiform discharges [23]. Brainstem auditory evoked potentials may be abnormal [23].

Patients surviving the acute neonatal crisis develop a pattern of cerebral palsy with spasticity and hypertonia [1, 3], though they may be hypotonic throughout infancy [3, 11]. Deep tendon reflexes are exaggerated and there is ankle clonus. A position of opisthotonos is common. The patient may be completely unaware of surroundings and have few spontaneous movements. There is no head control or other evidence of psychomotor development, such as sitting or rolling over, and no adaptive or social behavior (Figures 23.3 and 23.4). Eye movements may be

Figure 23.3 DG: An eight-month-old boy with nonketotic hyperglycinemia, in the tonic neck posture.

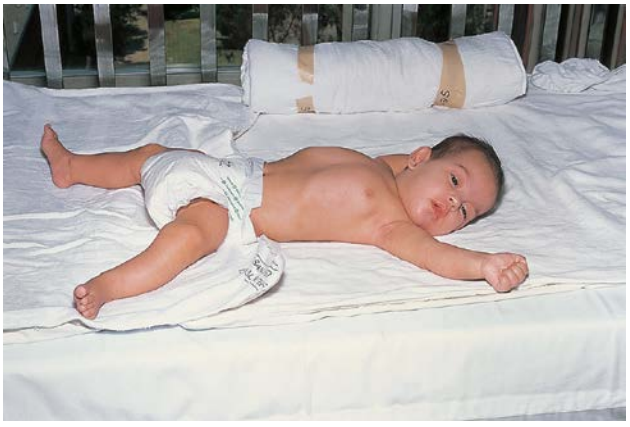

Figure 23.4 MC: An almost two-year-old with nonketotic hyperglycinemia. He survived a neonatal requirement for assisted ventilation following treatment with sodium benzoate but had little development or awareness of his environment. A feeding tube was required for nutrition. EEG revealed almost continuous seizure activity.

disconjugate. Gastroesophageal reflux develops frequently, and gavage or gastrostomy feeding may be required.

The natural history of the disease was recorded through two clinical surveys covering close to 150 patients [24, 25]. Nonketotic hyperglycinemia is heterogeneous and, while the majority of patients display the classic phenotype, a small number has been reported in whom a variety of milder or attenuated forms have been observed. Children with classical nonketotic hyperglycinemia often succumb early with a median age of death of 31.5 months, but can survive into their twenties [24, 25]. More girls than boys died in the newborn period; mean age of female death was less than one month, while that of boys was 2.6 years. Patients with the attenuated course mostly live. At the extreme from the classic, three affected girls [26] had only mild impairment; only one of the three was in an institution. Other families have been reported in which there was mild developmental delay [27–32]. Acute febrile illness has been associated with involuntary movements, paresis of upward gaze, and delirium. Severe mental impairment and seizures may be found despite an atypical late onset [32].

Up to 19 percent of nonketotic hyperglycinemia patients with neonatal presentation and 50 percent of patients with infantile presentation have an attenuated clinical course [24, 25], but the distinction between these two groups is somewhat artificial and influenced by time and the aggressiveness of treatment. Some patients can be classified as intermediary between these two groups. A milder course manifests with a better recovery in the first two months of life with increased wakefulness and no development of spasticity in infancy. There are few or no seizures, usually controlled by benzoate therapy and/or by a single anticonvulsant medication. More prominent than seizures are hyperactivity, behavioral problems, chorea and intermittent episodes of lethargy and ataxia. Most of these patients make developmental progress but remain mentally retarded, with developmental quotients varying between 20 and 70. Some boys became able to walk. There is evidence for a lower CSF/plasma glycine ratio and/or less severe mutations with residual activity in such milder affected patients [33]. Unfortunately, neither a lower CSF/plasma glycine ratio nor a mutation with residual activity nor a later onset of symptoms guarantees an attenuated disease course. However, a high CSF glycine (>240 μmol/L), brain malformations on cerebral imaging, a glycine peak on nuclear magnetic resonance imaging (MRI) or biallelic null mutations always lead to severe classical disease.

Patients with variant nonketotic hyperglycinemia can have severe or mild disease. Symptoms, in addition to a presentation like classical nonketotic hyperglycinemia and/or a mitochondrial disorder, can be leukoencephalopathy, optic atrophy, cardiomyopathy, pulmonary hypertension, lactic acidosis, and regression. We encountered a boy with muscular hypotonia, mild developmental delay and deceleration of head growth at the age of 17 months when attacks of recurrent vomiting started. From the age of 19 months, he rapidly deteriorated in crises with disturbed consciousness usually triggered by intercurrent illnesses. Ataxia and severe hypotonia developed (Figure 23.5A and B). The boy died at the age of 25 months. Plasma glycine was mostly normal, while CSF glycine was markedly elevated at 38 μmol/L resulting in pathological ratios of 0.12–0.16. In addition to severe and progressing leukoencephalopathy (Figure 23.6) laboratory investigations revealed elevations in lactate (4–5 mmol/L) and pyruvate (220 μmol/L). Reduced enzymatic activities of the glycine cleavage enzyme were found in a liver biopsy as well as of the PDH complex, specifically the E3 subunit,

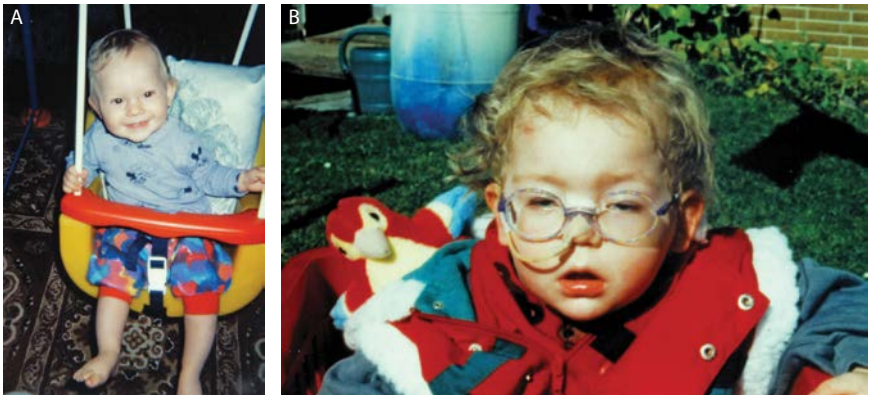

Figure 23.5 KR: A boy with variant nonketotic hyperglycinemia at the age of 12 months (A), still asymptomatic, and at age of 24 months (B), shortly before his death.

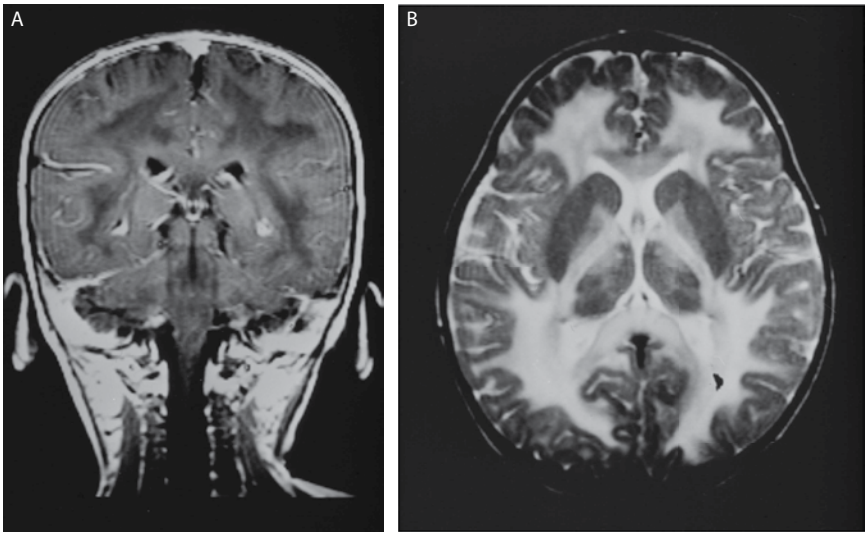

Figure 23.6 MRI of the brain of the patient in Figure 23.5 at the age of 18 months (A) displaying an extended almost symmetrical demyelinisation of the hemispheric medullary layer involving also the cerebellum; at 24 months (B) there was total demyelinization.

in muscle. Next generation sequencing revealed mutations in NFU1, a protein participating in the biogenesis of the Fe-S cluster [34]. This is the sulfur donor in the synthesis of lipoic acid, which becomes covalently attached to specific lysine residues of mitochondrial enzymes, among them the H protein of the glycine cleavage system.

We have again encountered such different presentations [35] as a neurodegenerative disease not unlike Tay-Sachs or Krabbe disease. The patient developed relatively normally for the first months of life and then, in the second half of the first year, showed progressive cerebral deterioration. This led to a state of decerebrate rigidity (Figure 23.7) followed by death.

MRI of the brain (Figure 23.8) [36–38], or computed tomography (CT), in this disease shows progressive atrophy and delayed myelination. The corpus callosum was abnormally thin in all patients and volume loss was both supra- and infratentorial. T_2-weighted images revealed decreased or absent myelination in the supratentorial white. These observations are consistent with reported neuropathology, including atrophy and corpus callosal thinning. Spongy rarefaction and vacuolation of myelin (Figure 23.9), as well as variable gliosis have been observed regularly [39, 40].

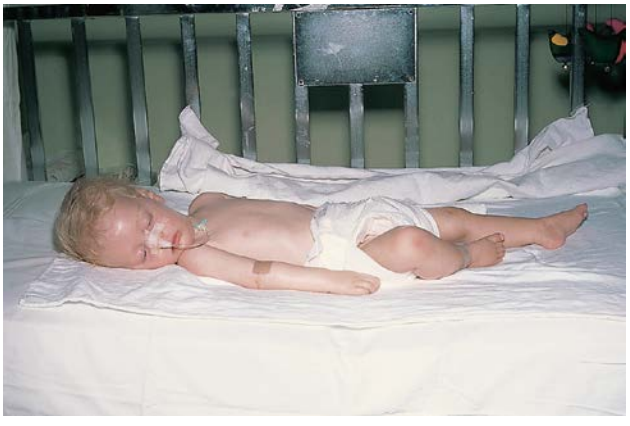

Figure 23.7 LS: At ten months. This patient presented with the picture of a cerebral degenerative disorder.

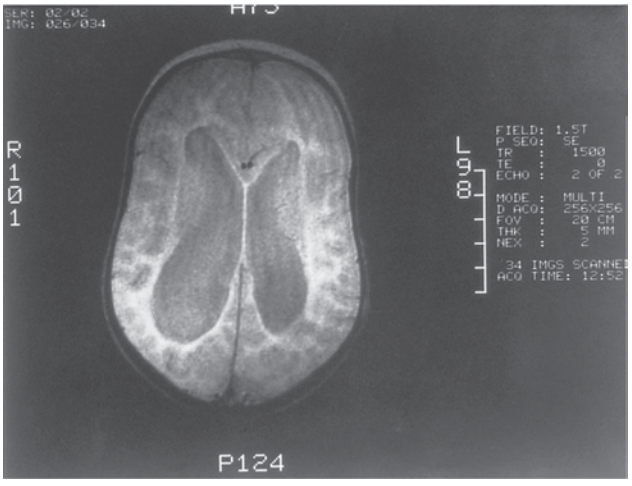

Figure 23.8 Magnetic resonance image of the brain of the patient in Figure 23.3. Dilated ventricles and sulci indicated a severe loss of volume of brain.

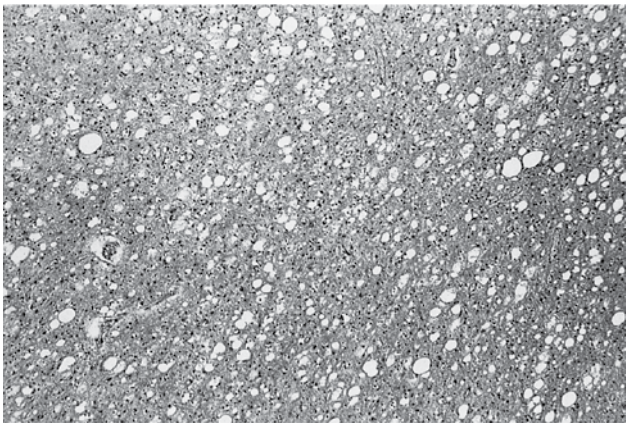

Figure 23.9 Histological section of the cortical white matter indicating the typical neuropathologic finding of spongy degeneration and gliosis.

Transient nonketotic hyperglycinemia represents a clinical presentation initially indistinguishable from the classic neonatal nonketotic hyperglycinemia [41]. The EEG may display the burst-suppression pattern, and the concentrations of glycine in plasma and CSF, and the CSF to plasma ratios may be diagnostic. Surprisingly, by 2–8 weeks of age, glycine levels have returned to normal. Normal cognitive and neurologic function at follow up has been reported but, in the patients followed by us, there was mild mental retardation at school age. Transient elevation of glycine in the CSF can be due to neonatal hypoxic ischemic injury and other severe neonatal brain insults [42]. In these patients, the glycine cleavage enzyme is not primarily affected and mutations are not found in the specific genes. In the initial phase, the lack of the pattern of lesions on diffusion-weighted brain images typical for nonketotic hyperglycinemia can give some help in the differential diagnosis.

GENETICS AND PATHOGENESIS

Deficiency of any of the components of the glycine cleavage is transmitted in autosomal recessive fashion [24, 25]. Defective activity of overall glycine cleavage was first described *in vivo* in studies of the metabolism of ^{14}C-labeled glycine [2]. Patients displayed virtually no conversion of glycine-1-^{14}C to respiratory $^{14}CO_2$ and the conversion of glycine-2-^{14}C to the third carbon of serine was similarly defective. Assay of the enzyme in the liver homogenates was reported in 1969 by Tada and colleagues [43]. The enzyme system is expressed in the liver, kidney, and brain, and until recently, could only be demonstrated in those tissues. Later, it has been shown that the system is induced in the transformation of B lymphocytes with Epstein–Barr virus [44]. This has turned out to be a convenient, but unreliable, approach to the diagnosis.

The glycine cleavage system (see Figure 23.1) is a mitochondrial complex with four individual protein components [4, 26, 45]. The P-protein is a pyridoxal phosphate-dependent glycine decarboxylase. The P- and H-proteins, lipoic acid-containing proteins, are required for the formation of CO_2 from glycine. All four are required for the conversion of glycine to CO_2, NH_3 and a one-carbon tetrahydrofolate (FH_4) derivative, which can then function in one carbon transfer, as in the formation of serine from glycine. The T-protein contains FH_4 and the L-protein is a lipoamide dehydrogenase.

Lipoic acid is required for the function of four mitochondrial enzymes; the E2 subunits of PDHC (see Chapter 50), α-ketoglutarate dehydrogenase, branched-chain α-ketoacid dehydrogenase (see Chapter 20), and the H-protein of the glycine cleavage system. Defects in the generation of lipoic acid variably affect these enzymes. Lipoate synthase has an iron sulfur cluster, which provides the sulfur atoms for the reaction. Multiple genes are involved in the biosynthesis of the iron sulfur clusters as well as of lipoic acid [6] and seven have, as yet, been proven as causes of variant nonketotic hyperglycinemia: LIPT2 and LIAS in lipoate synthase and glutaredoxin 5, IBA57, BOLA3, ISCA2 and NFU1 in the biosynthesis of the iron sulfur clusters.

Analysis of hepatic activity of the glycine cleavage system in 30 patients in Sendai [7, 46, 47] revealed undetectable levels in the classic disease and some residual activity in more indolent patients. Analysis of the protein component of the complex revealed that 87 percent had abnormalities in the P-protein, and this included all of the classic patients. Four patients had defects in the T-protein. In seven patients in whom the brain enzyme was assayed, the same component as in the liver was found to be defective. Another study demonstrated deficiency of the P-protein in approximately 80 percent of patients, of the T-protein in 15 percent of patients, and of the H-protein in 5 percent of patients, the latter patients likely represent variant nonketotic hyperglycinemia [6, 9]. Defective lipoylation was documented in some patients with a defect supposed in the H-protein [48]. Patients with a defect in the T-protein usually show about 20 to 25 percent residual activity, likely due to cycling over the P-protein [47, 49]. Immunochemical studies in patients with the classic presentation revealed virtually no P-protein [50].

Molecular studies of mutation have revealed heterogeneity for the gene of the P-protein, as well as for the T-protein. The P-protein gene [7, 10] has been located on chromosome 9p13-23 [51], as first suggested by a patient with nonketotic hyperglycinemia and the 9p- syndrome [52]. It contains 25 exons over 135 kb. The T-protein gene maps to chromosome 3p21.2-21.1 [53]. It has 6 kb over nine exons. The gene for the H-protein codes for a precursor protein of 173 amino acids and a mature protein of 125 amino acids [54]. The H-protein was mapped to chromosome 16q24 [55]. It spans 13.5 kb and contains five exons. The gene for the L-protein is unknown at present.

Nonketotic hyperglycinemia is present worldwide with an estimated incidence of one in 60,000 births. It is common in some Arabic tribes in Israel [56] and also in northern Finland where it occurs in one of 12,000 births [57]. This severe form of the disease was found to result from a point mutation of a G to T at nucleotide 1691 resulting in a change from serine to leucine at residue number 564 of the P-protein [58]. The absence of this mutation in 20 non-Finnish alleles and its presence in ten unrelated Finnish patients indicates the presence of a founder effect. Both this mutation and the Japanese deletion occurred in a region of the protein that is thought to be important for enzyme activity and for the binding of pyridoxal phosphate (Figure 23.10). In contrast, a patient with late onset nonketotic hyperglycinemia had a methionine to isoleucine change at position 391 in a very different part of the molecule [7]. In a comprehensive screening for mutations, Kure *et al.* [10] identified P- or T- protein mutations in 75 percent of neonatal and 83 percent of infantile families. No H-protein mutations were found. In 16 of 36 families, mutations were found on only one allele. Seven missense mutations were clustered in exon 19 at the cofactor binding site Lys754; a large deletion in exon 1 was

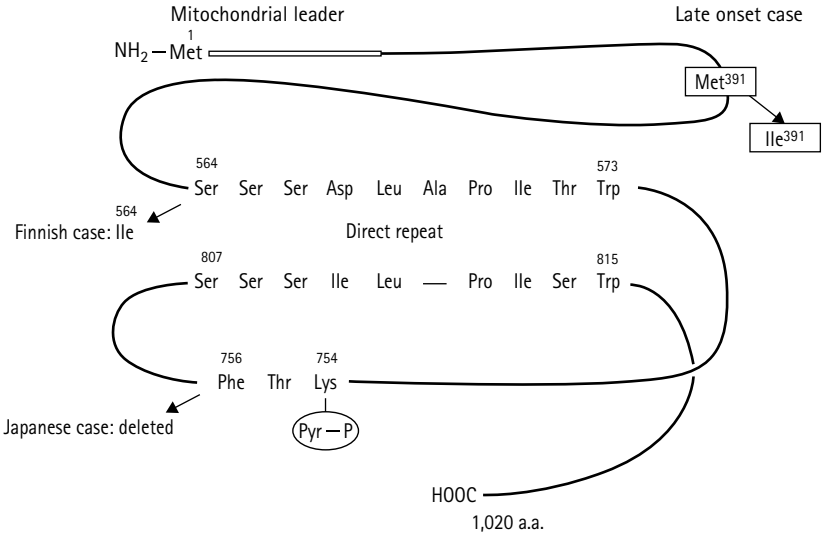

Figure 23.10 Molecular alterations in three different forms of the P protein of the glycine cleavage enzyme. The Japanese and Finnish phenotypes were of the classic type, while the patient with the methionine to isoleucine change at 391 had a more indolent disease. (Reprinted with kind permission from Springer Science & Business Media [7].)

found in Caucasian, Asian, and Black families with multiple origins indicated by haplotype analysis. Exonic deletions were present in 20 percent of mutations in the *GLDC* gene [59]. In addition to the most common deletion involving exons 1 and 2, deletions of all exons and of highly variable size have been seen as well as a single exonic duplication. Exonic deletions or duplications are usually not recognized on sequencing, but a combination of Sanger sequencing and complementary techniques such as MLPA or more recently *in situ* hybridization identify over 97 percent of variants.

Mutations in the gene for the T protein have included a G to A transition coding for a glycine to aspartic acid substitution at 269 (G269D) in a patient with classic neonatal disease [8], an A to G change leading to a histidine to arginine substitution at 42 (H42R) in an Israeli-Arab family [60], a deletion (183delC) causing a frameshift, and a G to C change causing D276H [10]. An atypical patient was a compound for two G to A changes, G47R and R320H. A novel splice-site mutation was found in three unrelated families [61].

About 5 percent of patients with nonketotic hyperglycinaemia do not have mutations in GLDC or AMT [62]. The majority of these patients have mutations in one of seven genes: *NFU1*, *BOLA3*, *ISCA2*, *LIAS*, *LIPT2*, *GLRX5* or *IBA57* [62–64]. A variety of missense and frameshift mutations have been reported. It is likely that additional genetic causes will still be identified in this group of patients [62].

Conventional approaches to genetic assistance to families are difficult in disorders in which the enzyme does not express in fibroblasts or amniocytes. Prenatal diagnosis of this disease became possible with the recognition that the cleavage system did express in chorionic villus samples. Thirty-one pregnancies were monitored [7], of which 23 were normal and eight affected. Similar results have been reported in experience with 50 pregnancies at risk [65], but 10 percent residual activity may make prenatal diagnosis inaccurate. Enzyme assay of cultured lymphoblasts has given intermediate results for heterozygote detection, but this approach may be inaccurate. Identification of the mutation permits prenatal diagnosis and detection of heterozygotes using molecular biology, and a prenatal diagnosis of the Finnish mutation has been made [58].

Concentrations of glycine are elevated in the blood, urine, and CSF. In spite of the large amounts of glycine found in the urine, it is possible to miss a patient with hyperglycinemia when screening the urine for amino acids by paper chromatography or electrophoresis. The normal glycine spot is very prominent. Also, patients are often studied when acutely ill, not eating, and being maintained on parenterally administered fluids. Under these circumstances, the excretion of glycine in hyperglycinemic patients may be normal. In general, it is better to screen for hyperglycinemia by assaying blood rather than urine. Blood concentrations are seldom brought into the normal range without vigorous benzoate therapy.

The concentrations of glycine are uniquely elevated in the CSF. Concentrations in classical patients have varied from 130 to 360 mmol/L [11, 24, 25, 66]. In the Finnish series of 19 patients, the mean was 93 mmol/L [67]. In control subjects, the concentration has generally been less than 12 mmol/L. The ratio of the CSF concentration to that of the plasma is substantially higher in patients with nonketotic hyperglycinemia than in hyperglycinemic patients with organic acidemia. In the Finnish series the mean was 0.11, whereas in control individuals the ratio was 0.02 (Table 23.1).

Table 23.1 Ratios of the concentration of glycine in the cerebrospinal fluid to that of plasma

Subject	Ratio
Finnish mean [67]	0.11
NKH, RH [11]	0.30
NKH, TZ [3]	0.10
Neurodegenerative variant [35]	0.07
Milder variant [29]	0.07
Control mean	0.02

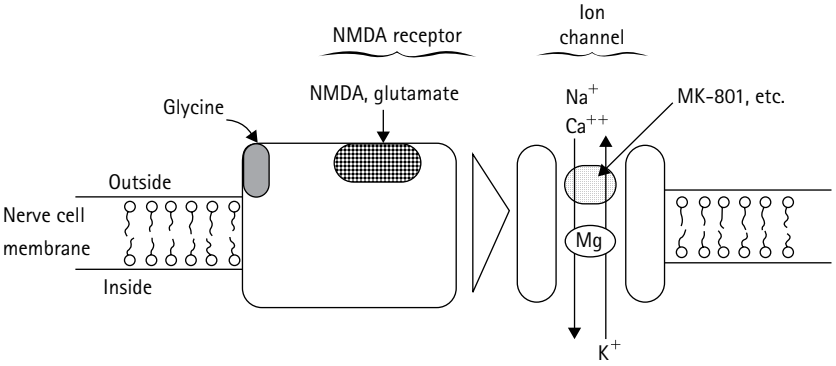

Figure 23.11 The N-methyl-D-aspartate receptor and the role of glycine. When glutamate or NMDA binds to the receptor the channel opens and positively charged ions flow to the nerve cell. Glycine binds at a different site on the receptor and acts as a facilitator.

It is important to recognize that the ratio may be meaningless if concentrations are normal. A diagnosis of hyperglycinemia requires an elevated level of glycine in plasma.

We have observed patients with milder degrees of clinical expression in whom the ratios, though abnormal, were less elevated than in the classic phenotype.

The exact pathophysiology of nonketotic hyperglycinemia is not yet understood. The activity of the glycine cleavage enzyme is high in liver, brain, and placenta. In the brain, it keeps glycine levels very low, resulting in a typically low CSF to plasma glycine ratio. Glycine is connected to multiple biochemical pathways. Via the formation of methylene-tetrahydrofolate, the glycine cleavage enzyme, in conjunction with the glycine-serine hydroxymethyltransferase, are among the main donors of one-carbon-group metabolites.

Glycine has long been known to be active in the nervous system, but older information on glycine as an inhibitory neurotransmitter at strychnine receptors never fitted with the picture of intractable seizures [68]. It does however explain the severe muscular hypotonia as well as neonatal apnea. It is now clear that glycine is also an excitatory neurotransmitter (Figure 23.11) with a profound influence on the N-methyl-D-aspartate (NMDA) receptor, which normally responds to the excitatory amino acid glutamate [69]. Glycine functions as an allosteric agonist permitting glutamate to be excitatory at much smaller contractions.

TREATMENT

The management of this disease is considerably less than satisfactory. Exchange transfusion or dialysis, or sodium benzoate, may be life-saving in the neonatal period and may permit weaning from the ventilator, but many families made aware of the grim prognosis prefer not to take these steps. The plasma concentrations of glycine may be lowered by the administration of sodium benzoate and even more by dietary restriction. It is now clear that treatment with large amounts of benzoate, which joins with glycine to form hippurate which is then excreted in the urine, can actually lower CSF concentration of glycine, and that there are dose–response relationships [70]. Patients so treated had a substantial decrease in seizures. Doses employed have ranged from 500 to 750 mg/kg per day for classical and 300 to 500 mg/kg/day for milder affected patients. The aim is to reduce plasma levels of glycine to 120 to 300 μmol/L. Overdosing of benzoate beyond the individually required dose has high morbidity and mortality [33]. The concentration of benzoate in serum increases exponentially when glycine becomes limited with low serum glycine levels. Concentrations of benzoate over 2.5 mM are associated with nausea, vomiting, and lethargy [71]. The clinical benefit of lowering glycine by benzoate has been convincingly documented in several studies. Most notable many patients with a milder clinical course become seizure free with only use of benzoate and dextromethorphan. Improved neurocognitive outcome is notable with best results in those consistently treated from the neonatal period [56, 72]. Nevertheless, developmental progress has often been disappointing, except in one patient who was reported as developmentally normal [73].

We and others [74] have added dextromethorphan to the benzoate regimen as a noncompetitive antagonist at the NMDA receptor. Dosage employed has been in the range of 5–22 mg/kg per day. Among the four patients reported from Baltimore [74, 75], very beneficial effects on development were observed in a patient treated from the first days of his life. This was less evident in our experience, and results were mixed in the other Baltimore patients, including one who died at 12 weeks. Similarly, mixed results were reported by others [72, 73]. Arnold *et al.* [76] pointed out that dextromethorphan is metabolized very differently in different individuals and that measuring levels is necessary to ensure adequate dosage. Anticonvulsant effects were associated with levels of 50–100 ng/mL. Cimetidine, an inhibitor of P450 activity, was found to increase dextromethorphan levels in a rapid metabolizing individual.

Benzoate treatment has been observed to lead to carnitine deficiency in patients with this disease [76, 77]. It would appear prudent to supplement patients under treatment with carnitine and to measure levels. It is also prudent to avoid treatment with valproate in this disease, because it causes increase in levels of glycine [78], inhibits the activity of the glycine cleavage system, and can result in severe worsening of symptoms [79].

There is currently no effective treatment known for variant nonketotic hyperglycinemia. Treatment with high dose lipoic acid has not been successful, either *in vitro* or *in vivo* [62–63].

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24

Serine deficiencies

MAJOR PHENOTYPIC EXPRESSION

Psychomotor retardation, microcephaly and seizures of neonatal or childhood origin, progressive polyneuropathy in adults, low concentrations of serine in blood and cerebrospinal fluid (CSF), and deficiency of 3-phosphogylcerate dehydrogenase (3-PGDH), 3-phosphoserineaminotransferase (PSAT), or phosphoserine-phosphatase (PSP). ASCT1 is the major transporter for serine in the central nervous system (CNS); its deficiency leads to neurologic abnormalities similar to those of defects in serine biosynthesis or transport. Therapy with serine may be helpful in any of the defects.

INTRODUCTION

Low concentration of serine in blood and CSF result from defective activity of each of three enzymes on the biosynthetic pathway for serine and lead to a neurologic phenotype. The serine deficiency syndrome was first described by Jaeken and colleagues in 1996 [1]. In these two patients, the disease was the result of deficiency in the first (3-PGDH) and last (PSP) steps in the biosynthesis of serine (Figure 24.1). The majority of patients described have been due to 3-PGDH deficiency. Few patients have had PSP or PSAT deficiency. In addition to its function in protein synthesis; L-serine is neurotropic and a precursor of phosphatidylserine and sphingomyelin, as well as glycine and D-serine.

Deficient activity of 3-PGDH was documented in fibroblasts, and mutations were found in the gene located on chromosome 1p12 [2, 3]. Mutational analysis has documented abnormalities in the PSAT gene [4]. In the only patient reported with PSP deficiency, the diagnosis was confirmed by assay of the enzyme and documentation of mutation [5].

CLINICAL ABNORMALITIES

The first two patients with 3-PGDH deficiency [1] had congenital microcephaly, postnatal retardation of growth, hypogonadism and hypertonia. Psychomotor retardation was profound, and they had seizures. One brother had bilateral congenital cataracts. Congenital microcephaly, seizures and severe mental retardation were commonly seen [2] in patients with this disease. A majority of patients with 3-PGDH deficiency had infantile onset of microcephaly, intractable seizures and severe developmental delay [2, 6–10]. A variety of seizures observed included infantile spasms, tonic-clonic seizures, atonic and gelastic seizures [11].

Difficulty with feeding, vomiting and irritability are common in these infants and crying may appear inconsolable [11]. Seizures usually begin within the early weeks or months of life. Hypertonia in infancy is followed by spastic tetraplegia. Adducted thumbs, inguinal and umbilical hernias, hypogonadism, and abnormal hair has been reported, as well as megaloblastic anemia [4, 7, 9, 11–15].

A patient [9] reported with PSAT deficiency seemed normal at birth but was admitted to hospital at two weeks for poor feeding and episodes of cyanosis. By seven weeks, he had jerking movements and seizures that were intractable despite anticonvulsant therapy. He had microcephaly. Neuroimaging revealed cerebral atrophy, a hypoplastic cerebellar vermis and poor white matter development. Magnetic resonance imaging (MRI) of the brain in 3-PGDH deficiency revealed atrophy with large ventricles and hypomyelination [13].

The patient with phosphoserine phosphatase deficiency [16] had pre- and postnatal retardation of growth, psychomotor retardation and features of Williams

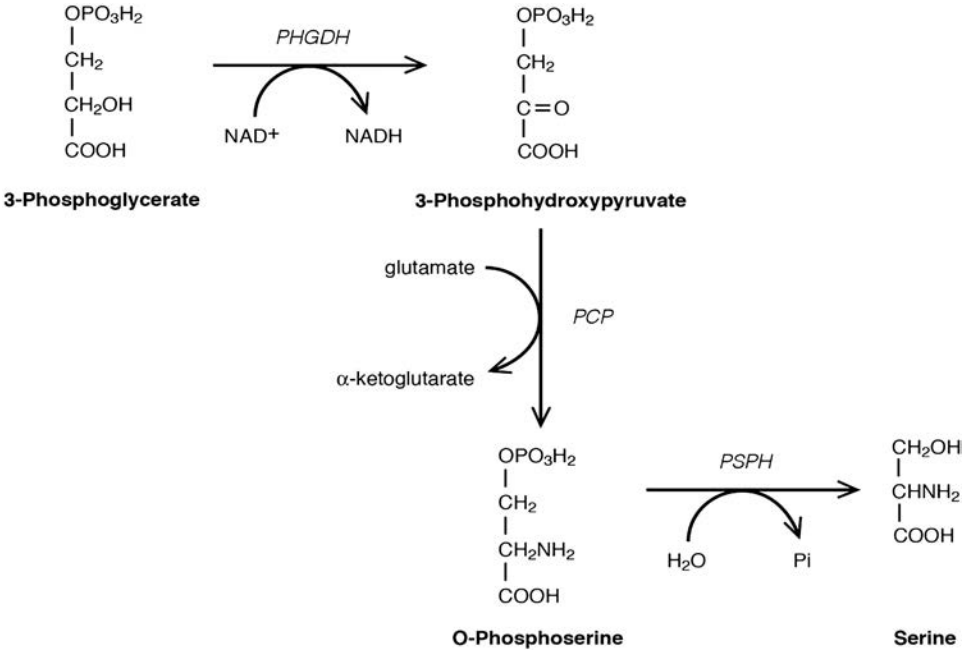

Figure 24.1 The biosynthesis of serine.

syndrome, which was considered to be unrelated, although both genes are on chromosome 7.

A milder phenotype of 3-PGDH deficiency in which absence seizures had a juvenile onset at five and nine years was described by Tabatabaie *et al.* [17]. Neither of two siblings were microcephalic, but they were delayed in psychomotor development with IQs of 50. MRI of the brain was normal.

A different adult phenotype was described by Meneret *et al.* [3]. The patient did have mild difficulties walking and mental retardation in childhood, along with congenital cataracts, but progressive axonal sensorimotor polyneuropathy began in adulthood. He also had mild cerebellar ataxia and nystagmus. At 31 years of age, he was diagnosed as having Charcot-Marie-Tooth disease. MRI of the brain showed T_2 hyperintensities, but not the hypomyelination of the infantile form.

The biochemical characteristic of the disease is a low concentration of serine in the CSF [18]. Concentrations in 3-PGDH deficiency have all been 13 μmol/L or lower. The 13 was in the adult phenotype patient; the rest were 6–11 μmol/L. Plasma concentrations of serine have been low or borderline. They have ranged from 28–64 μmol/L in 3-PGDH deficiency. In PSAT deficiency, the CSF serine ranged from 5–18 μmol/L, and in PSAT deficiency it was 18 μmol/L. Plasma concentrations of serine may be normal; so examination of the CSF amino acids is the diagnostic gold standard. Plasma concentrations, unlike those of the CSF, are influenced by feeding. Also, there are age-specific ranges, with highest concentrations of serine found in the newborn period [19].

Neu-Laxova syndrome is a lethal disease with multiple congenital anomalies [21] patients have microcephaly, distinctive facial features, ichthyosis and malformations of the brain including lissencephaly and cerebellar hypoplasia. It may result from mutations in any of the three enzymes of serine biosynthesis [22].

Serine racemase converts L-serine to D-serine, which is a neuromodulator binding to and activating the N-Methyl-D-aspartate (NMDA) subtype of glutamate receptors [20].

Assay for the enzyme in cultured fibroblasts of patients with 3-PGDH deficiency has revealed considerable residual activity, ranging from 12 to 25 percent of the lower range of reference values [18]. Enzyme assay in the index patient with PSAT deficiency was inconclusive [4]; mutational analysis made the diagnosis. In PSP, deficiency enzyme assay was definitive [5].

GENETICS AND PATHOGENESIS

All of the serine deficiency diseases are inherited in an autosomal recessive pattern.

In PGDH deficiency, mutations have been found throughout the gene on chromosome 1p12 [6, 19]. Phenotype cannot as yet be predicted from either enzyme or mutational analysis [11].

In PSAT deficiency, analysis for mutations appears to be required to establish the specific diagnosis. In 12 patients with PSP deficiency, a microdeletion was found at 7q11.23 which encompassed the elastin gene, accounting for the Williams syndrome [16].

Information is not available on pathogenesis, but serine is a precursor of phospholipids and glycolipids, important constituents of the CNS. The existence of these three defects in the biosynthetic pathway indicates that adequate amounts of serine are important for the development and function of the nervous system.

TREATMENT

The logical treatment of this disease was begun by Jaeken [6]. A dose of 200 mg/kg of L-serine led to improvement in seizures. Higher doses, 500 mg/kg and the addition of glycine (200 mg/kg) were then followed by clinical and biochemical improvement [7]. Response of irritability and cessation of inconsolable crying began in 2–3 weeks, and in some, seizures completely disappeared [11]. EEG became normal. With a dose of 400 mg/kg in one patient, head growth was arrested and there were infantile spasms, and hypsarrhythmia. Increases to 600 mg/kg and 700 mg/kg led to normal head growth and EEG. This (500–700 mg/kg) is the currently recommended dose. Failure of improvement in many patients has been attributed to intra-uterine deficiency of serine and its effect on prenatal development [19].

Prenatal therapy of 3-PGDH deficiency prevented an onset of neurologic manifestations for 12 years at report [11, 15].

Limited experience with treatment of PSP deficiency indicated some catch up in head growth. Successful treatment of PSAT deficiency has not been reported.

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PART 3

HYPERAMMONEMIA AND DISORDERS OF THE UREA CYCLE

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25

Introduction to hyperammonemia and disorders of the urea cycle

INTRODUCTION

The central function of the urea cycle is the irreversible detoxification of ammonia to urea. Distinct disorders involve each of the six enzymes of the cycle (Figure 25.1) [1, 2]. These include deficiencies of the mitochondrial matrix enzymes *N*-acetylglutamate synthase (EC 2.3.1.1; MIM 237310; prevalence <1:2,000,000), ornithine transcarbamylase (OTCD; EC 2.1.3.3; MIM 311250; 1:56,000) (Chapter 26), and carbamoyl phosphate synthetase (CPSD; EC 6.3.5.5; MIM 237300; 1:300,000) (Chapter 27) or the cytosolic enzymes argininosuccinate synthetase 1 (citrullinemia type 1; EC 6.3.4.5; MIM 215700; 1:250,000; Chapter 28), argininosuccinate lyase (argininosuccinic aciduria; EC 4.3.2.1; MIM 207900; 1:218,000; Chapter 29), and arginase 1 (argininemia; EC 3.5.3.1; MIM 207800; 1:950,000; Chapter 30), as well as deficiencies of the aspartate glutamate carrier citrin (citrullinemia type 2, frequent in East Asians; MIM 605814, <1:2,000,000 outside China and Japan) and the mitochondrial ornithine transporter 1 (HHH [hyperammonemia, hyperornithinemia, and homocitrullinuria] syndrome; MIM 238970; <1:2,000,000;

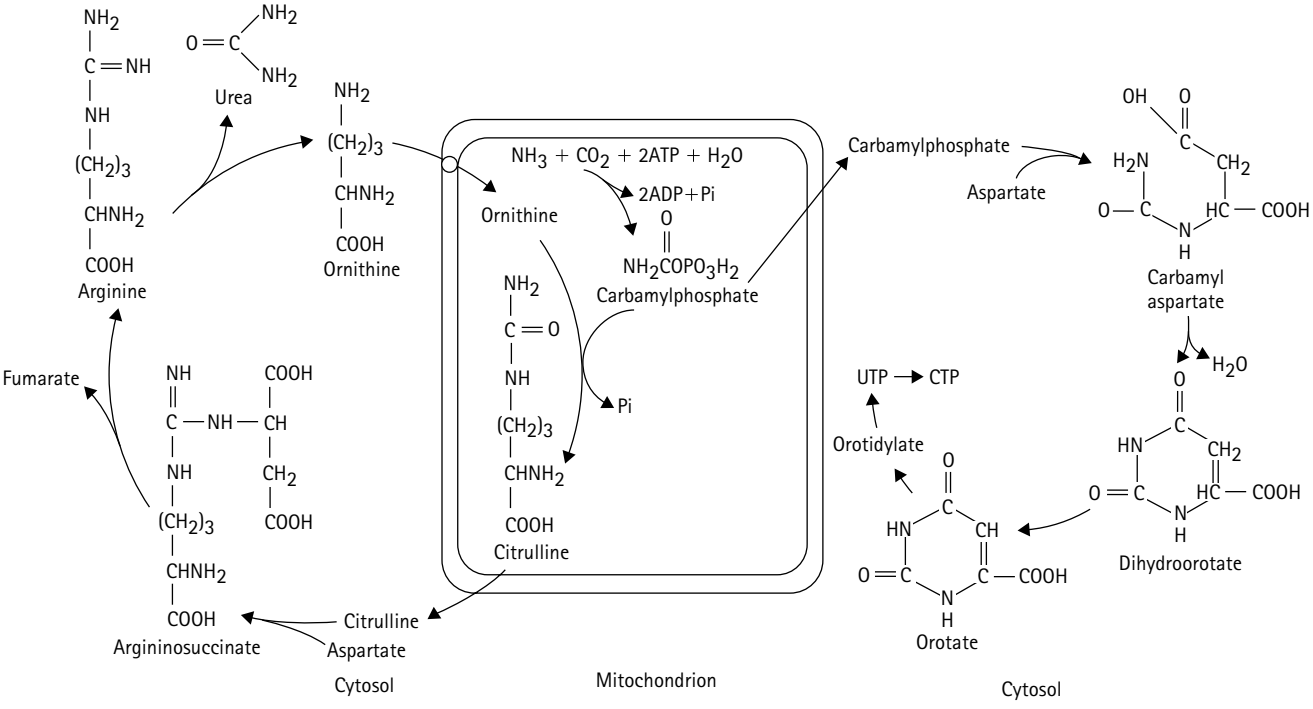

Figure 25.1 The urea cycle.

Chapter 31). In addition, there is a syndrome of transient hyperammonemia of the newborn [3], in which the early clinical manifestations mimic those of the severe defects of urea cycle enzymes and may be fatal, but if the patient can get through the first 5 days of life, the problem disappears and prognosis is good. Mitochondrial carbonic anhydrase VA deficiency [4] and lysinuric protein intolerance (Chapter 32) are also associated with episodic hyperammonemia, but the major expression of the latter is distinct with extreme failure to thrive.

Estimates based on multicenter studies and data from newborn screening indicate prevalences for all urea cycle disorders (UCDs) to be 1:35,000 newborns with large variation among single diseases in the USA. Similar estimates have been calculated for Finland, Japan, and Germany. True prevalence is probably much higher. It can be suspected that a significant proportion of cases remain undiagnosed.

Patients with UCDs present with a wide spectrum of clinical disease and severity, as a function of different exogenous triggers, genetic variability, residual enzyme activity and the resulting impact on ammonia production and detoxification. Except for argininemia, citrin deficiency and lysinuric protein intolerance, the most severe and critical disease manifestation is (neonatal) hyperammonemic encephalopathy resulting in death or severe neurologic and mental impairment. This classic presentation of acute neonatal life-threatening coma is one of the most dangerous and difficult to manage constellations in metabolic medicine (Figure 25.2), but acute life-threatening encephalopathy can also develop later at any time in life. Lethargy leads to a state of complete unresponsiveness reminiscent of surgical anesthesia. Breathing stops, and in the absence of intubation and artificial ventilation, death ensues. This clinical picture and concentration of ammonia from 400 to 2000 μmol/L are seen in a variety of disorders in addition to those listed above. These include the organic acidemias and the disorders of fatty acid oxidation. Effective management requires a precise diagnosis, but vigorous therapy should be initiated immediately on recognition of the hyperammonemia.

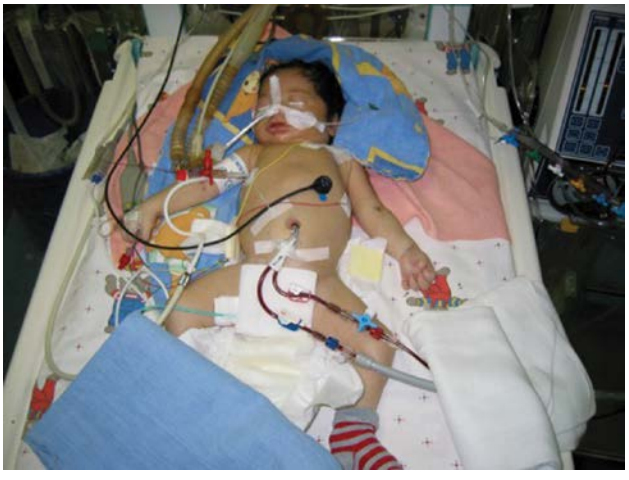

Figure 25.2 Emergency therapy of an infant in hyperammonemic coma including continuous veno-venous hemodiaflitration.

WORK UP OF THE PATIENTS WITH HYPERAMMONEMIA

A systematic approach to the work up of an infant in hyperammonemic coma is shown in Figure 25.3. Timely differential diagnosis is important because different disorders require very different treatments. It must proceed with dispatch in order to institute appropriate therapy. Initial studies can be carried out in any clinical laboratory and provide direction to the next diagnostic and therapeutic steps. Further studies must be carried out in a laboratory that specializes in biochemical genetic analysis in order to make a precise definitive diagnosis. Liver biopsy and enzymatic analysis may be required for the final diagnosis of ornithine transcarbamylase deficiency, carbamoyl phosphate synthetase deficiency, or *N*-acetylglutamate synthetase deficiency; but mutational analysis provides a less invasive approach that mostly, but not always, provides the definitive diagnosis. In every infant in unexplained coma, the blood concentration of ammonia should be measured. A work up for hyperammonemia should be undertaken in any newborn with an ammonia concentration greater than 150 μmol/L and in any older infant and adult at values over 100 μmol/L. The serum concentrations of bicarbonate, sodium, and chloride are measured, the anion gap assessed, and the urine tested for ketones. The presence of acidosis and an anion gap, or massive ketosis, indicates that hyperammonemia is due to one of the organic acidemias. These disorders include propionic acidemia (Chapter 2), methylmalonic acidemia (Chapter 3), isovaleric acidemia (Chapter 8), and glutaric aciduria type II (Chapter 45). Specific diagnosis is made by organic acid analysis of the urine or the acylcarnitine profile of the blood.

Hyperammonemia may also be seen in acute exacerbation of disorders of fatty acid oxidation. These episodes are characteristically hypoketotic. They are usually associated with lactic acidosis and/or hypoglycemia, raising the possibility of a diagnosis of Reye syndrome, but we have seen an acute hyperammonemic episode in a teenage girl who turned out to have medium chain acyl-CoA dehydrogenase deficiency (Chapter 39) that met all the criteria for a diagnosis of ornithine transcarbamylase (OTC) deficiency, except that when the liver was biopsied, its OTC activity was normal [5]. Nowadays, patients affected with disorders of fatty acid oxidation are diagnosed in extended newborn screening programs.

Hyperammonemic patients who do not have an organic acidemia are seldom acidotic. If there is an abnormality in acid-base balance in a patient with an urea cycle defect, it is more likely to be a respiratory alkalosis, though apnea and hypoxia can lead to lactic acidosis; so

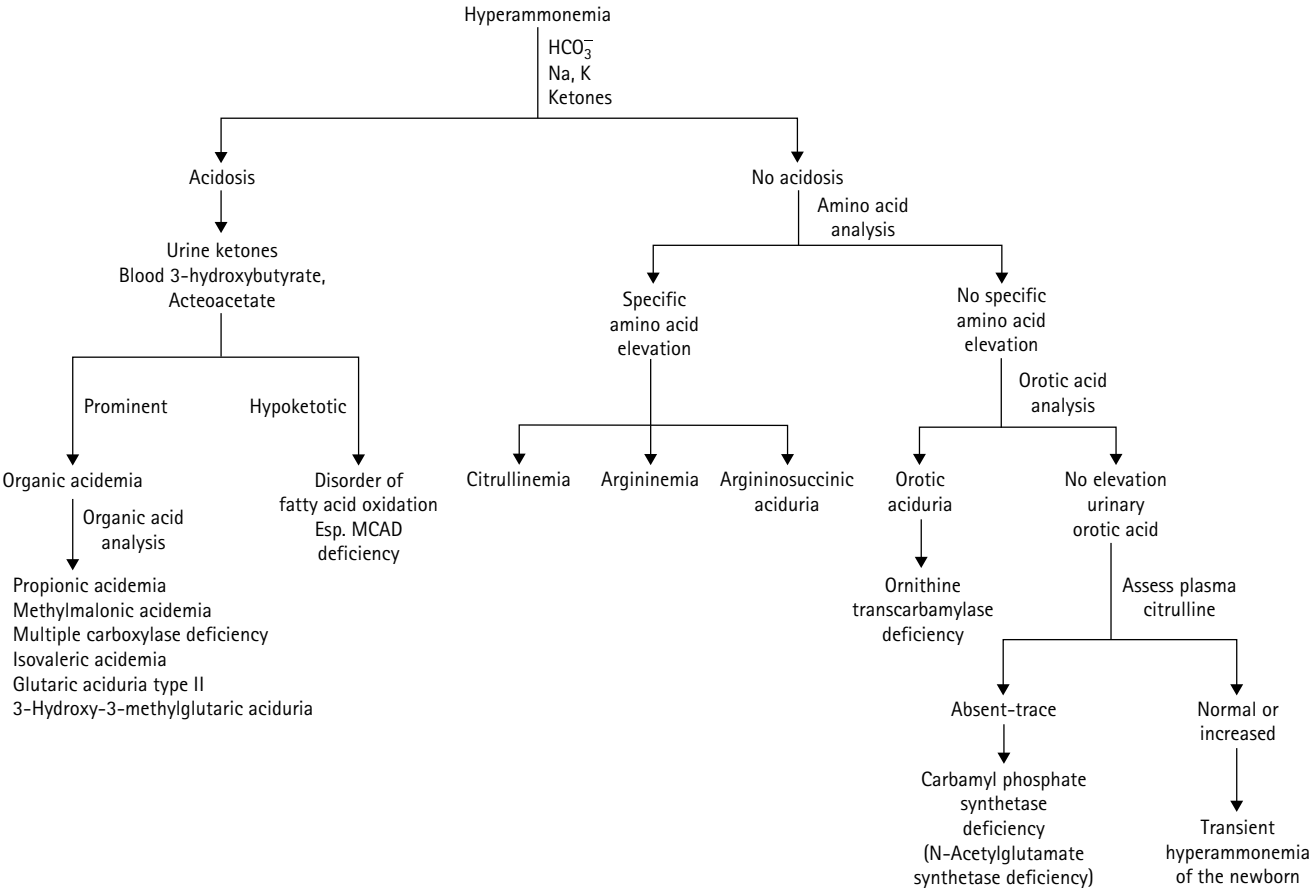

Figure 25.3 Diagnostic work up of the hyperammonemic infant in coma.

adequate oxygenation and perfusion should be assured before these assessments are made. The next step toward definitive differential diagnosis is the quantitative assay of the concentrations of amino acids in blood and urine. Plasma amino acid patterns are distinct in the UCDs, with elevated concentrations of glutamine, alanine, and mostly asparagine in all, and low concentrations of citrulline and arginine in deficiencies of *N*-acetylglutamate synthase, OTCD, CPSD and mitochondrial carbonic anhydrase VA deficiency. Assessment of the plasma amino acids will provide a definitive diagnosis in patients with argininemia and citrullinemia, whereas urine is required in argininosuccinic aciduria (Chapter 29). Citrulline is the product of carbamylphosphate synthetase 1 and ornithine transcarbamylase activity, and the substrate for argininosuccinic synthetase (Figure 25.1). Thus, its concentration is absent or markedly reduced in the four proximal defects, markedly elevated in citrullinemia type 1 and argininosuccinic aciduria and only slightly above normal in argininemia. In citrullinemia, concentrations of citrulline in plasma usually exceed 1000 μmol/L. They are elevated to levels of 50–250 μmol/L in argininosuccinic aciduria, and can also be slightly raised to 54±22 μmol/L in transient hyperammonemia of the newborn [3]. The normal range is 6–20 μmol/L. For distinguishing CPSD from OTCD, it is crucial to determine urinary orotic acid which is elevated in OTCD and decreased or normal in CPSD. A deficiency of N-acetylglutamate synthase, the enzyme needed for the production of the cofactor N-acetylglutamate for carbamylphosphate synthetase has the same constellation of metabolites as CPSD. The mitochondrial carbonic anhydrase VA deficiency which is also associated with low-normal orotic acid excretion is also very similar [4]. Carbonic anhydrase 5A provides bicarbonate to CPS, pyruvate carboxylase, propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase.

Diagnosis in older children and adults with partial deficiencies may be less straightforward than in neonates. During symptomatic episodes, plasma ammonia concentrations may be in the range of 150 to 250 μmol/L, rather than greater than 500 μmol/L, and normal when the patient is clinically stable. Citrulline and arginine concentrations are often low–normal in partial proximal defects, rather than absent-trace. Definitive diagnosis in these cases is best attempted by molecular testing to detect specific mutations. The genes for OTCD, CPSD, N-acetylglutamate synthase, and mitochondrial carbonic anhydrase VA should be sequentially investigated.

In older children and adults, a number of acquired disorders can also present with hyperammonemia, including liver disease, Reye syndrome, drug toxicity, and hepatotoxin ingestion. History, prothrombin time,

a urinary toxic screen, and plasma amino acid pattern should help to differentiate these disorders. Symptomatic hyperammonemia may also result from a urinary tract infection in which the infecting Proteus mirabilis has urease activity, which produces ammonia from urea. A patient with prune-belly syndrome and massive dilatation of the urinary tract developed coma with a blood concentration of ammonia of 140 μmol/L (202 μg/dL).

Concentrations of glutamine are regularly elevated in patients with UCDs, and concentrations of alanine are also usually elevated, while concentrations of aspartic acid are elevated in some patients. These findings are nonspecific but potentially helpful in diagnosis, as sometimes an elevated level of glutamine is found in a patient who had not been expected to have hyperammonemia. While concentrations of ammonia may vary from hour to hour, the elevated concentration of glutamine signifies a state in which there has been a more chronic oversupply of ammonia. The transamination of pyruvic acid and that of oxalacetic acid and the subsequent amidation of glutamic acid all represent detoxification mechanisms in the attempt to handle excessive quantities of ammonia. This contrasts with hyperammonemia due to other inherited metabolic diseases such as the organic acidemias, which are not associated with a primary urea cycle defect and usually have normal glutamine, arginine and citrulline concentrations.

Amino acid analysis is not diagnostic in OTCD and CPSD, but in both of these conditions, levels of citrulline and arginine may be low. They are distinguished by measurement of the excretion of orotic acid, which is increased in OTCD, but not in CPSD, deficiency of N-acetylglutamate synthetase or mitochondrial carbonic anhydrase VA [4, 6]. In OTCD, this is a reflection of the accumulation of carbamylphosphate, which then leaves the mitochondria and follows the cytosolic pathway of pyrimidine synthesis (Figure 25.1). There may be so much orotic acid in the urine that it forms a white crystalline precipitate. In mice with OTCD, calculi are found in the bladder [6, 7].

Overproduction of pyrimidines leads to the presence of large amounts of uracil and increased amounts of uridine in the urine. In most patients with OTCD, orotic aciduria is always present. However, we have reported one patient [6] and studied others with partial variants in whom orotic acid excretion was not present when they were clinically well and could not be induced by means of a protein load, but was readily evident at the time of illness induced by infection.

TREATMENT OF HYPERAMMONEMIA

The treatment of the patient with hyperammonemia has many common features relevant to states of elevated ammonia, regardless of cause. Therapy must not be delayed because coma duration of less than 1.5 days [8] and timely start of treatment are the most important determinants of outcome. Specialized pediatric hospitals should have first-line medications, consensus-based treatment-protocols and should act according or similar to the following principles of a recently published guideline [9]:

- Nothing by mouth. Stop protein intake. Immediately start infusion of 10% glucose.
- Tailor i.v. fluid and glucose substitution (see Table 25.1).
- Start first-line medication (see Table 25.2).
- Collect plasma and urine for diagnostic purposes without delaying therapy.

In the acute hyperammonemic onset usually seen in infancy, all intake of protein or other sources of nitrogen is stopped. Initially, the dietary emergency regimen might be protein-free but reintroduction of protein or essential amino acids should be considered after 24 to 48 hours and started once blood ammonia level has fallen to <100 μmol/L. In addition to close control of laboratory parameters such as

Table 25.1 Consensus-based treatment protocol for pediatric (specialized) hospitals treating patients with unknown acute hyperammonemia according to *Suggested guidelines for the diagnosis and management of urea cycle disorders* [9]

		Protein, liquid and glucose management				
Escalation level	NH_3 (μmol/L)	Protein	Liquid i.v. (ml/kg/d)	Glucose i.v. (mg/kg/min)	Insulin	Comments[e]
1	<100	Stop[a]	100–150[b]	10[c]	i.n.[d]	–
2	100–250	Stop[a]	100–150[b]	10[c]	i.n.[d]	Inform metabolic clinic!
3	250–500	Stop[a]	100–150[b]	≥10[c]	i.n.[d]	Inform dialysis clinic!
4	>500	Stop[a]	100–150[b]	≥10[c]	i.n.[d]	Hemodialysis!

[a] Stop protein intake for 24 hours (maximum 48 hours).
[b] Liquid management might be adapted to hospital- and age-specific requirements.
[c] Glucose management might be adapted to age-specific requirements.
[d] If necessary (blood glucose >150 mg%) give 0.05 units/kg/h.
[e] Hyperglycemia can be dangerous, monitor every 0.5–1 hour.
Monitor blood ammonia levels every 3 hours.
Monitor electrolytes, blood gases and lactate regularly, e.g. every 3 hours.

Table 25.2 Further consensus-based treatment protocol for pediatric (specialized) hospitals treating patients with unknown acute hyperammonemia according to *Suggested guidelines for the diagnosis and management of urea cycle disorders* [9]

First-line medication in the initial episode before definitive diagnosis[e]								
		Sodiumbenzoate i.v.[d]		Sodiumbenzoate/-phenylacetate (Ammonul®) i.v.[d]		L-Arginine hydrochloride 21% i.v.[d]		
Escalation level	NH_3 (μmol/L)	Bolus (mg/kg) in 90–120 min	Maintenance (mg/kg/d)[c]	Bolus (mg/kg) in 90–120 min	Maintenance (mg/kg/d)[c]	Bolus (mg/kg) in 90–120 min	Maintenance (mg/kg/d)	Carbamyl-glutamate p.o.
1	<100	–	–	–	–	–	–	–
2[a]	100–250	250	250–500 5.5 g/m²/d[b]	250	250–500	250–400	250	Bolus: 100 mg/kg Maintenance: 25–62.5 mg/kg every 6 h
3[a]	250–500	250	250–500 5.5 g/m²/d[b]	250	250–500	250–400	250	
4[a]	>500	250	250–500 5.5 g/m²/d[b]	250	250–500	250–400	250	

[a] Consider L-carnitine i.v. 100 mg/kg, hydroxycobalamin i.v./i.m. 1 mg and biotin i.v./p.o. 10 mg.
[b] If patient >20 kg body weight.
[c] If on dialysis maintenance doses should increase to 350 mg/kg/d.
[d] Higher doses of arginine may be employed in patients with citrullinemia and arginosuccinic aciduria and none in argininemia. First-line medications must be diluted in 10% glucose prior to i.v. application. (Caution: L-Arginine-HCl may cause metabolic acidosis and extravasation may lead to tissue necrosis) Zofran may be given as an antiemetic (0.15 mg/kg i.v.) during the first 15 minutes of the priming infusion.
[e] Control electrolytes due to possible danger of hypernatremia and hypokalemia.

ammonia, electrolytes, and glucose, plasma amino acids must be determined and the results available daily. Water and electrolytes are provided intravenously, and anabolism is promoted by the administration of glucose. Pharmacologic approaches to therapy include the provision of arginine to keep the urea cycle supplied with sufficient ornithine to keep it running and the provision of alternate pathways for the excretion of waste nitrogen, such as sodium benzoate and sodium phenylacetate/-butyrate. Extracorporeal methods, such as hemodialysis, are often required in the acutely hyperammonemic newborn. The most effective treatment of the acute hyperammonemic crisis that occurs in the classic neonatal disease is continuous veno-venous hemodiaflitration [8–11]. Exchange transfusion is not an effective modality in such an infant, but it may reduce levels sufficiently to buy some time while the hemodialysis is being prepared, and it has been effective in some patients with transient hyperammonemia of the newborn [3]. Peritoneal dialysis is sometimes recommended for hyperammonemic patients, and we would agree that it is effective in an older infant, child, or adult with hyperammonemia but, in our experience, it has been unsatisfactory in the neonatal period [12]. Hemodiaflitration and hemodialysis have been shown to be more effective than exchange transfusion, peritoneal dialysis or arteriovenous hemofiltration, but the logistics are such in most hospitals that this modality can seldom be mobilized promptly to meet the needs of a newly diagnosed newborn. This is an argument for early transport of such an infant to an institution with experience in the rescue of such infants. Even though continuous veno-venous hemodiaflitration is the optimal modality for extracorporeal ammonium detoxification [8], prognosis is not related to dialysis modality but primarily to the duration of coma before the start of treatment confirming the necessity for rapid and aggressive management.

An advance in management has been the application of extracorporeal membrane oxygenation (ECMO) in the treatment of the hyperammonemic neonate [13]. A number of patients with various urea cycle disorders have received partial or total orthotopic liver transplants to provide enzyme replacement or somatic gene therapy, respectively [14]. In all successful cases, this has cured the hyperammonemia and permitted a normal protein intake. However, its effectiveness is hampered by expense, limited availability of donor organs, and significant morbidity and mortality from complication of transplantation or immunosuppression. Liver transplantation does not normalize citrulline concentrations, which is primarily produced in the intestine; thus, following transplantation, supplementation with citrulline or arginine may need to be continued. Ideally, orthotopic liver transplantation should be carried out between 3–6 and 12 months of age before irreversible neurologic damage has occurred, in patients with severe neonatal-onset disease, and patients suffering from recurrent severe decompensations despite intensive medical treatment [9]. New trends and emerging therapies include the use of hypothermia in neonatal hyperammonemia [15] and hepatocyte transplantation as a therapeutic bridging option in UCD patients awaiting liver transplantation [16].

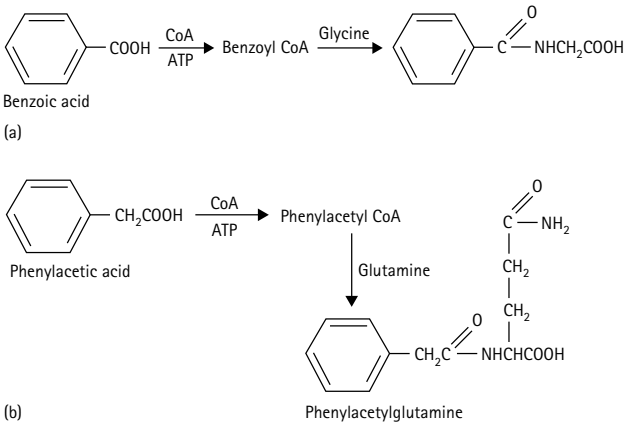

Figure 25.4 Structures of benzoic and phenylacetic acids and the mechanism of their excretion of waste nitrogen.

The pharmacologic approach to the provision of alternate methods of waste nitrogen excretion represents a major advance in the management of hyperammonemia (Table 25.2 and Figure 25.4) [9, 13, 17, 18]. The principle is that benzoate is effectively conjugated with glycine to form hippurate, which is efficiently excreted in the urine, and similarly phenylacetate is conjugated to form phenylacetylglutamine, and this compound is excreted in the urine; both provide pathways for getting rid of nitrogen that cannot be excreted as urea and would otherwise accumulate as ammonia. These measures have been employed along with exchange transfusion or peritoneal dialysis [3], but we have found pharmacologic therapy effective in patients in whom exchange transfusion and peritoneal dialysis were having little or no effect [6, 13].

Intravenous benzoate and phenylacetate are available commercially as a mixture from Ucyclid Pharma (Phoenix, AZ, USA). In Europe, most patients are treated with intravenous benzoate. Along with benzoate and/or phenylacetate, arginine is infused. This provides an essential amino acid in a patient with a complete block in arginine synthesis and provides ornithine substrate for any patient in whom there is not a complete block at carbamylphosphate synthetase or ornithine transcarbamylase. It may be particularly useful as an effector of acetylglutamate synthetase and hence activator of carbamylphosphate synthetase. It is particularly useful in patients with citrullinemia (Chapter 28) or argininosuccinic aciduria (Chapter 29). Supplies of benzoate, phenylacetate, and arginine should ideally be kept on hand and available to the neonatal intensive care unit in anticipation of the diagnosis of such an infant. Often, a patient is first recognized in a community hospital where benzoate and phenylacetate are not available, but arginine is. The arginine infusion should be started and the patient then transported to the tertiary care center. We have found that many patients will respond to arginine alone. This is often true in citrullinemia or argininosuccinic aciduria, but is also true of patients with OTCD in which a variant enzyme permits activity of the urea cycle as long as the cycle does not run out of ornithine substrate. Females with OTCD, with one normal X chromosome seldom completely inactivated, can respond to arginine alone. The effect of arginine as an activator of carbamylphosphate synthetase should also be salubrious for patients with OTCD, citrullinemia, and argininosuccinic aciduria. Operationally, for arginine as well as benzoate and/or phenylacetate, a priming infusion is followed by a regimen of continuous infusion until the ammonia has reached the normal range (Table 25.2). The use of mannitol for cerebral edema may also provide nitrogen excretion through diuresis in patients treated with benzoate, phenylacetate, and arginine.

The pharmacologic regimen is started on diagnosis in the acute neonatal hyperammonemic crisis and can be pursued while the dialysis team is being assembled. It may be effective in obviating the need for dialysis. It is usually effective in the management of recurrent episodes of hyperammonemia that occur in patients under therapy [9, 17] at times of infection or other cause of catabolism, or vomiting, leading to an inability to continue oral treatment, because therapy of these episodes is generally initiated more promptly. It is also true that many patients successfully treated initially have died in later episodes of intercurrent hyperammonemia. The ideal approach to management is very early diagnosis so that hyperammonemia is prevented or treated before there is major elevation of the serum concentration of ammonia.

The drugs are supplied as concentrated solutions which would cause hyperosmolarity if infused directly. They are diluted in 30 mL/kg of 10% glucose for the priming infusion, e.g. 0.25–0.4 g/kg of arginine hydrochloride (2.5–4 mL/kg of 10% arginine HCl), and 0.25 g/kg of sodium benzoate and 0.25 mg/kg sodium phenylacetate in approximately 30 mL/kg of 10% glucose over 1–2 hours. Later, the drugs are diluted in the 24-hour maintenance fluids, which are also 10% with respect to glucose, providing extra calories to spare catabolism. When the sensorium clears, and there is no vomiting, further calories can be provided by nasogastric tube in the form of polycose or Ross product Prophree or Mead Johnson product PDF usually diluted to supply 0.7 kcal/mL. Nitrogen intake is not resumed until the hyperammonemia has receded.

As the main concept for long-term management catabolism must be avoided as much as possible. In addition to intercurrent illnesses, especially if associated with high fever and decreased intake of food and fluids, very dangerous triggers are severe exercise, seizures, trauma, burns, steroid administration, chemotherapy, and gastrointestinal hemorrhage. Furthermore, drugs – especially steroids, valproate, haloperidol and L-asparaginase/pegaspargase – and the postpartum period (due to catabolism and the involution of the uterus) are important triggers for late-onset hyperammonemia [9].

The infant or child usually requires a combination of pharmacologic therapy and restriction of the intake of protein. Arginine is employed in doses of 0.1–0.2 g/kg, 0.2–0.3 g/kg or even higher in citrullinemia type 1 or

argininosuccinic aciduria. In OTCD and CPSD, sodium benzoate is given in doses of 0.25(–0.5) g/kg/day, and citrulline, which is more palatable than arginine, is employed as a source of ornithine in a dose of 0.17–0.25 g/kg. Oral sodium phenylbutyrate has been employed as a source of phenylacetate in doses of 0.25 g/kg. It may be used as a substitute for benzoate, but many of our patients have found it unpalatable. A gastrostomy may be required. Glycerol phenylbutyrate (RAVICTI®) has the same mechanism of action as sodium phenylbutyrate, but is a sodium- and sugar-free prepro-drug of phenylacetic acid that has little odor or taste. Also, phenylbutyrate may deplete branched chain amino acids levels and cause menstrual dysfunction/amenorrhea in up to 25% of post-pubertal females [9]. To avoid complications, e.g. mucositis or gastritis, sodium benzoate and sodium phenylbutyrate should be administered several times daily during meals with abundant fluids [9]. Acute toxicity of benzoate and phenylbutyrate has been rare, but severe overdoses (2 to 10 times recommended) have led to symptoms that may be clinically mistaken for hyperammonemic episodes, including lethargy, hyperventilation, metabolic acidosis, cardiopulmonary collapse, and death [18].

Dietary treatment is an essential anchor point of long-term management and requires the knowledge of a specialist metabolic dietitian. For infants and older children, nutritional management involves the use of a high-caloric, low-protein diet supplemented with essential amino acids and, if necessary, vitamins and minerals. This is most readily accomplished by using small amounts of natural protein, an essential amino acids formula, and supplemental calories provided by a formula that does not contain protein. Protein is generally restricted to 0.7 g/kg and supplemented with 0.7 g/kg of a mixture of essential amino acids. We have felt that the optimal intake of whole protein should be determined in each patient and have found supplementation with relatively small amounts of alanine to be effective [19], but in urea cycle defects, extra essential amino acids are often necessary. The FAO/WHO/UNO 2007 Report (summarized in [9]) can be used as age- and gender-dependent recommendations for energy intakes. Especially in young infants and children, fasts should be avoided and snacks given to reduce the possibility of (overnight) catabolism. Periodic measurement of plasma amino acids (which include glutamine) and blood ammonia may permit adjustment of therapy before clinical symptoms appear.

Chronic management of patients with urea cycle defects has also been effective using mixtures of the keto and hydroxy-acid analogs of essential amino acids [20]. These mixtures are no longer available in the United States, but there may still be a place for this anabolic approach to the removal of nitrogen, and long-term therapy in which keto acids were combined with benzoate in the successful treatment of a 30-month-old infant who at report was developing normally [15]. In this patient, nocturnal gavage was useful in the administration of the keto acids, arginine and benzoate.

N-acetylglutamate is an essential cofactor of the carbamoyl phosphate synthase enzyme, and a small number of patients have been found with N-acetylglutamate synthase deficiency [21]. Carbamylglutamate is a structural analog of N-acetylglutamate, and Santiago Grisolía first suggested its use in hyperammonemia, caused by N-acetylglutamate synthetase deficiency (Nyhan, personal communication). This has turned out to be the case; treatment reduced levels of ammonia in the blood [22] and increased the incorporation of ^{15}N-ammonium chloride into urea [23]. Carbamylglutamate has also been used successfully to treat the acute hyperammonemia of methylmalonic and propionic acidemia [24]. Inhibition of N-acetylglutamate synthase is the mechanism by which propionylCoA and similar compounds cause hyperammonemia [25].

Carbamylglutamate is available in Europe in 200 mg tablets for oral use, which limits its availability in acute hyperammonemic coma. It has been used via nasogastric tube. In the acute situation, a dose of 100 mg is appropriate. In the chronic management of N-acetylglutamate synthetase deficiency, doses of 10–100 mg/kg per day have been effective.

Prior to the development of alternate pathway therapy using ammonia scavengers (e.g. sodium benzoate, sodium phenylacetate/-butyrate), virtually all children with neonatal UCDs died in the newborn period or during infancy. Between the 1980s and mid-1990s, the one-year survival rate for children with early-onset type UCD was approximately 50% and worse for early-onset CPS1D [26]. This has slowly changed with the widespread availability of ammonia measurement in hospitals, growing knowledge about the disease and the use of alternate pathway therapy. Nowadays, the one-year survival rate for early-onset and late-onset UCD patients has substantially improved [27]. A recent study demonstrated that the early-onset disease manifestation, a peak-blood ammonia level >1000 μmol/L and coma on admission are associated with the highest risk of mortality during a hyperammonemic episode [28]. Infants who are more than 5 days in hyperammonemic coma are invariably handicapped developmentally.

Long-term morbidity remains substantial in UCD patients. It could be shown that 50% of patients with UCDs suffer from intellectual disability [29]. These data were confirmed by a more recent study including 103 subjects with neonatal-onset UCDs [30]. However, uncertainty with regard to the impact of peak-blood ammonia level on neurocognitive outcome in UCDs exists. While Bachmann suggested that neurocognitive outcome does essentially depend on the initial peak-blood ammonia level [31], surprisingly Ah Mew and coworkers could not correlate the peak-blood ammonia level with poor cognitive outcome [30].

In a compilation of the Paris experience with the management and outcome of urea cycle defects [32], there were 121 patients with neonatal presentations, 66 of them OTC-deficient males, reflecting the severity of disease in this population in that all but one died promptly. Overall

mortality of those with neonatal presentations was 84%. Girls with neonatal presentations of OTC deficiency also died in the neonatal period.

Of 96 late onset forms, OTC deficiency was also the most common. In this group, 18% died in the initial episode, but none once the diagnosis was established. It is clear from this experience that late-onset forms are not uncommon. Neurologic manifestations were common in all the survivors.

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26

Ornithine transcarbamylase deficiency

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal hyperammonemic coma in the male and varying expression in the female, consistent with X-linked transmission; convulsions; elevated concentrations of glutamine and alanine, decreased arginine and citrulline; orotic aciduria; and defective activity of hepatic ornithine transcarbamylase.

INTRODUCTION

Ornithine transcarbamylase deficiency (OTCD) is the most common inherited disorder of the urea cycle. The most classic of the infantile urea cycle presentations is that of OTCD in the male. Onset is in the neonatal period with coma and/or convulsions, and in the absence of effective intervention, it is rapidly fatal. A sizable number of males have variant enzymes and a milder and later presentation, but hyperammonemia can still be fatal, even in adulthood. Females who have two X chromosomes have varying phenotypes mainly depending on the proportion of active and inactive X chromosomes.

The enzyme ornithine transcarbamylase (OTC) or ornithine carbamoyltransferase (EC 2.1.33) is the second enzyme in the urea cycle (see Figure 25.1) and found almost exclusively in liver. There is less activity in the small intestine and a small amount in the brain. It catalyzes the conversion of ornithine and carbamylphosphate to citrulline. The hepatic enzyme is located in the mitochondria [1]. The enzyme is a trimer in which identical subunits have a molecular weight of about 38 kDa [2, 3]. The OTC enzyme is synthesized in the cytosol and transported to its mitochondrial site of activity. The protein synthesized contains an *N*-terminal signal peptide that is specifically recognized by a receptor complex in the outer mitochondrial membrane [4]. After translocation across the membranes, proteolytic processing by two peptidases yields the mature protein [5]. Once imported into the mitochondria, the OTC subunits require folding to form the active trimer, and this process is mediated by molecular chaperones.

Defective activity of the enzyme is readily demonstrable in a biopsied liver [6, 7]. The gene on the X-chromosome codes for the precursor protein that is imported after translation into mitochondria. The human OTC precursor cDNA has been isolated and cloned [8]. It is localized to band p21.1 in the short arm of the X chromosome [9], just proximal to the locus for Duchenne muscular dystrophy. The genes for glycerol kinase, adrenal insufficiency, chronic granulomatous disease, Norrie disease, and retinitis pigmentosa are all in this area, and a number of contiguous gene syndromes have resulted from deletions. Patients with these large deletions may have up to five severe genetic diseases making them extremely difficult to manage [10]. Large deletions within the OTC gene account for about 16 percent of mutations in affected males [11]. Another 10 percent have point mutations in a *TaqI* recognition site in exon 5 in (TCGA) which changes the code for arginine at position 109 of the mature protein to either glutamine or a stop codon and reduces enzyme activity to one percent of normal or less [12, 13]. Many other mostly private point mutations have established an enormous heterogeneity. By 2006, more than 350 mutations had been documented [13].

CLINICAL ABNORMALITIES

OTCD in its presentation in the neonatal male infant (Figure 26.1) provides the classic picture of a defect in

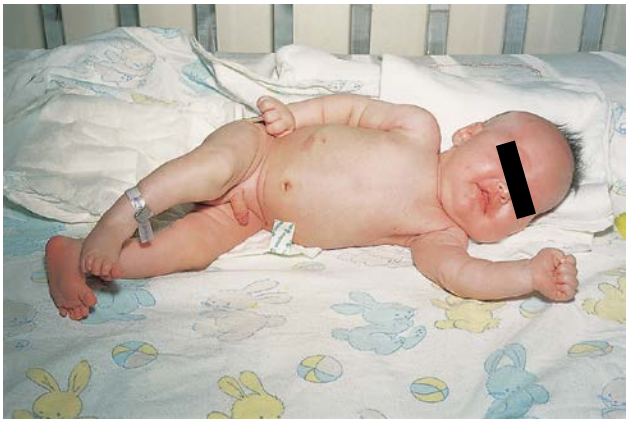

Figure 26.1 JN: A male infant with OTCD after recovery from neonatal hyperammonemia. The site of a Tenckoff catheter that had been unsuccessfully used for peritoneal dialysis is evident on the abdomen.

the urea cycle (see Figure 25.1) [14]. According to new data, this accounts for approximately one third of all patients respectively 50 percent of males with this disease [15–17]; however, neonatal-onset male patients might be underrepresented in statistical analyses because of early and undiagnosed death. Prior to the development of pharmacologic approaches to the removal of waste nitrogen using benzoate and phenylacetate, this disorder was always fatal and usually within just a few days of birth. Until today mortality and morbidity are still high.

Affected infants are often thought to have sepsis. Occasionally, the diagnosis of hyperammonemia is made once blood culture is found to be negative. On the other hand, we have encountered neonates with urea cycle defects who have actually had sepsis, further confusing the diagnosis. It is, therefore, advisable to obtain blood for ammonia in any infant in coma. A bulging fontanel may suggest intracranial hemorrhage, but it is more often the result of cerebral edema, and computed tomography (CT) scan should resolve the issue.

The infant appears normal at birth and remains so during a period of hiatus, which may be as short as a few hours and is seldom longer than 48 hours. The infant then begins to be lethargic and to refuse feedings. Vomiting and hypotonia may be observed. Grunting or rapid respirations may occur, and there may be a respiratory alkalosis. Neurologic findings may include increased deep tendon reflexes and papilledema. Convulsions may be generalized, and the electroencephalogram (EEG) is usually abnormal. The infant may have hypertonia, but there is progression to a deep coma that is indistinguishable from surgical anesthesia. Ultimately, the infant stops breathing, and unless intubated and artificially ventilated, he dies. Despite initial improvement following dialysis or other interventions, which decrease ammonia concentrations, most of these patients have died [17]. Those surviving because of successful pharmacologic therapy have usually had severely impaired mental development, if the initial hyperammonemic coma has been profound and prolonged. Most have had recurrent hyperammonemic crises at times of catabolism induced by intercurrent illness, and each further episode appears to worsen the prognosis for mental development. If, in a family at risk, the diagnosis can be made prior to hyperammonemic coma and the patient prevented from ever having such an episode, then the development of the nervous system should be normal, but patients fitting these criteria among males with the classic form of OTCD are very rare. In a few successfully managed patients, there were subsequent early liver transplantations, which prevent further metabolic decompensations. Patients who survive the neonatal attack remain at high risk of dying in infancy in a subsequent hyperammonemic episode that accompanies an acute infection.

The major metabolic characteristic of patients with OTCD is hyperammonemia. Levels found in the classic neonatal form of the disease are usually over 700 μmol/L (1000 μg/dL). Coma is generally present when the concentration exceeds 250 μmol/L (400 μg/dL). In infants dying of the disease, levels may range from 400 to 1700 μmol/L (600–2500 μg/dL). In the presence of levels over 300 μmol/L (500 μg/dL), one sees fixed dilated pupils and complete apnea. Cerebral edema occurs in some patients at these levels. Normal neonatal ammonia is <100 μmol/L, but levels up to 180 can be observed in sick infants without an inherited metabolic disease. Problems in obtaining and handling blood samples invariably raise levels, and exercise such as squeezing a ball, can also raise the level to 150 μmol/L [18]. Thus, a normal level eliminates hyperammonemia, but an abnormal level that does not fit with clinical findings may have to be repeated or confirmed by the presence of an elevated glutamine. Consensus on which specific neonatal level should prompt intravenous (IV) therapy has not been reached or whether mild elevations of ammonia in chronically treated patients should be treated intravenously [18], with the current discussion converging towards an accepted consensus; see the *Suggested guidelines for the diagnosis and management of urea cycle disorders* [19] (Chapter 25).

In the female, the range of variation in symptomatology is especially great (Figure 26.2) [15, 17, 20–24]. This is consistent with varying degrees of inactivation of the normal X chromosome called for by lyonization as a random process. A balanced translocation involving an X chromosome in a female can result in the deletion of the OTC locus and cause consistent, nonrandom inactivation of the X-chromosome with the normal OTC gene leading to complete OTC deficiency and severe phenotype [25]. There is a broad spectrum of presentations after the newborn period even within the same family, with some individuals developing hyperammonemic episodes in infancy or early childhood, others in later childhood, and still others not until adulthood with pregnancy and diet changes constituting some of the precipitating causes. Among these patients, delay in diagnosis is common. In a series of 13, the mean interval from onset of symptoms to

Figure 26.2 TC: A two-year-old girl with OTCD. During infancy, she had many episodes of hyperammonemia despite therapy with arginine, benzoate, and phenylacetate, but each was treated promptly, and cognitive development was good. Nevertheless, she died in a subsequent hyperammonemic episode.

diagnosis was 16 months, and the range was 1–142 months [20]. At one end of the spectrum, is a small number of female infants with an overwhelming clinical picture indistinguishable from that of the male and taking the same clinical course [17].

Over 90 per cent of the female patients follow a somewhat less severe disease course often with episodes of recurrent hyperammonemia but still death in childhood. Others have had recurrent nausea and vomiting beginning in infancy or as late as nine years of age. This condition should be included in the differential diagnosis of cyclic vomiting. Attacks may be accompanied by headache, slurring of speech, or screaming. An attack may also present with ataxia. A patient with recurrent episodes of intense headache and ataxia may appear to have migraine. During the attack, the patient may display muscular rigidity or opisthotonus. There may be convulsions. Some chronic hyperammonemic symptoms appear to be age-specific. In infants, episodes of vomiting, feeding problems, hepatopathy and recurrent neurologic symptoms (e.g. lethargy, irritability, psychomotor/mental retardation, movement disorder, and convulsions) are common. In older children and adults, behavioral abnormalities (e.g. biting, self-injury, nocturnal restlessness, hyperactivity) and psychiatric signs (e.g. confusion, irritability, agitation, headache, and aggression) can be found. Recently, it has been demonstrated that late-onset patients most often presented with progressive mental retardation, movement disorders and epilepsy [24]. Especially in adults, symptoms may mimic specific psychiatric or neurologic disorders [24, 26]. In such patients, chronic hyperammonemic symptoms normally precede an acute metabolic decompensation and are often, unfortunately, only identified retrospectively. Stroke-like episodes have also been described [26]. Triggers for acute metabolic manifestation of late-onset OTCD include switching from low-protein breast milk to high-protein formula or cow's milk, fever, infections, vomiting, gastrointestinal bleedings, decreased energy or protein intake, and surgery. Importantly, drugs, especially valproate, steroids, haloperidol and L-asparaginase/pegaspargase, and the postpartum period (due to catabolism and the involution of the uterus) are important trigger factors for late-onset hyperammonemia [19, 27].

Any patient with OTCD and symptomatic attacks of hyperammonemia may develop coma, and this may lead to death. Hepatomegaly is seen in some patients, and there may be abnormalities in liver function tests. In an older child seen for the first time with hyperammonemia, these findings may be thought at first to represent primary disease of the liver. OTCD was, on occasion, initially diagnosed as Reye syndrome [21]. Attacks may be precipitated by a large intake of protein, infection, surgery, or immunization. Impaired mental development may be progressive with further episodes. Some patients may present with acute liver disease leading to failure [28]. Over the years, many patients develop variable hepatic fibrosis and focal changes resembling glycogen storage disorder and cirrhosis [24]. Hepatocellular carcinoma has been described [29]. Disorders of fatty acid oxidation may have an identical Reye-like presentation and meet all of the conditions for a diagnosis of OTCD, except that the enzyme activity of the liver is normal [30].

At the other end of the spectrum are women who are completely asymptomatic. They are found to be heterozygous for deficiency of OTC because a male son or other relative is found to have the classic disease. Some of these women have a dislike of protein foods and thus have not stressed the system. Others appear to have no trouble with protein. Nevertheless, a careful study of the IQ scores of heterozygotes, identified by the urinary excretion of orotic acid following a protein load, were found to be six to 10 points lower than in the controls, who were relatives in whom the orotic acid test was negative [23].

Some males have been reported in whom there was a much milder or late-onset clinical phenotype, similar to that described in females. These patients appear to represent variants different from the classic one in that they have a defect in the enzyme that leads to partial activity [24, 31–35]. Prominent symptoms are recurrent vomiting, lethargy, irritability, and protein avoidance. A patient with one of these variants may have normal development and may progress normally in school. The disease may present in adulthood [33]. It may present with bizarre behavior [33, 34]. In fact, recurrent episodes of bizarre behavior may be the only symptoms of this type of OTCD [33]. Measurement of orotic acid and orotidine, even after allopurinol or a protein load, failed to elucidate the diagnosis in this patient,

but a high protein diet led to orotic aciduria. In the late onset male, as in the symptomatic female, the disease is nevertheless potentially lethal, and death may ultimately occur in a hyperammonemic episode even after a number of symptom-free years. In a series of 21 male patients who presented at ages ranging from two months to 44 years, 43 percent died [35]. The mean interval from the age at onset to that at diagnosis was 8.8 months, with a range of up to 54 months. An initial diagnosis of Reye syndrome was made in 52 percent of the patients. Death with cerebral edema was the result of an initial episode following relatively trivial surgery in a 52-year-old man [36]. The diagnosis was made by mutational analysis in his heterozygous daughter. Prenatal diagnosis of her affected twin sons led to effective management. Among complications of OTC deficiency, sudden strokes have been reported [26]. This has also been seen in carbamylphosphate synthetase (CPS) deficiency, citrullinemia type I, and an enlarging group of metabolic diseases.

The diagnosis is suspected on the basis of the blood level of ammonia and suspicions are confirmed by elevations in glutamine and often alanine. An algorithmic approach to the exclusion of nonurea cycle causes of hyperammonemia and the differentiation among specific urea cycle defects (Chapter 25) identifies those disorders in which a specific amino acid, such as citrulline, is elevated. Elevated excretion of orotic acid then differentiates OTCD from that of CPS in which orotic acid levels are normal. A deficiency of N-acetylglutamate synthase, the enzyme needed for the production of the cofactor N-acetylglutamate for CPS, is also associated with low-normal orotic acid excretion. Finally, the mitochondrial carbonic anhydrase VA deficiency which is also associated with low-normal orotic acid excretion is very similar [37]. Definitive diagnosis of OTCD usually requires assay of the enzyme in a biopsied liver, but mutation analysis when positive obviates this. If the mutation in a family is known, analysis of the DNA will make the diagnosis, but mutational analysis may also be successful in the absence of a family mutation.

GENETICS AND PATHOGENESIS

The gene for OTC is located on the X chromosome [8, 9]. The disease is expressed as an X-linked dominant. Thus, in females in whom a major proportion of cells contain the inactivated normal X chromosome, the severity of disease may be as great as in the homozygous male. At the other end of the spectrum, even asymptomatic female heterozygotes may have lower IQs than their homozygous normal relatives [23].

The molecular defect in OTC is readily detected in the liver. This enzyme is also expressed in intestinal mucosa, and therefore the diagnosis has been made by assay of tissue obtained by rectal or duodenal biopsy [38]. However, the gold standard is the assay of the biopsied liver.

In males with the lethal neonatal disease, enzyme activity is virtually absent [6, 7]. In males with the partial variants, levels range from 5 to 25 percent of normal. Some of these patients have been reported to have virtually zero activity [39], but this is not likely to reflect the level of activity that is functional *in vivo*. Abnormal proteins tend to be unstable and break down readily under conditions of *in vitro* assays. In symptomatic heterozygous females, levels of activity have ranged from 4 to 25 percent of normal [7]. As much as 97 percent of normal activity has been found in known heterozygous mothers of affected children. Because the female is a mosaic of hepatocytes, the level of activity found in a biopsy may not necessarily reflect the *in vivo* activity of their conglomerate, but the diagnosis should nevertheless be clear. In some males with partial variants, the kinetic properties of the enzyme have been studied [40–42]. These variant enzymes have been found to have alterations in the Km for ornithine or carbamylphosphate or the optimal pH. The use of antibody prepared against the normal human enzyme has shown no cross-reacting material (CRM) in most hemizygous males, but a few were CRM-positive [42].

The cloning of the gene for OTC [8] and the elucidation of its structure [43] have permitted the identification of more than 350 different mutations [13, 44–46]. Mutations have been distributed across the entire gene. Some 42 percent of mutations were associated with acute neonatal diseases; 21 percent were in later onset males [44] and 37 percent in manifesting females. The incidence of new mutation in the genesis of girls with OTCD has been much greater than in boys [46], suggesting that mutation is more common in sperm than in ova. Among males, only 7 percent had sporadic mutations, while more than 90 percent inherited their mutations from their mothers. Among females, only 20 percent inherited their mutations from their mothers, whereas 80 percent have spontaneous mutations or inherited mutations from the father's mutated sperm. Therefore, the chance of a mother having a second affected child depends on whether she has previously had an affected male or female. A mother found not to carry the mutant allele in a child could have gonadal mosaicism. This was found in a somatically normal woman who produced multiple affected males [47]. Fortunately, this is very rare.

A very great amount of heterogeneity has been identified. Most families have had unique mutations. Large deletions of an exon or more were found in 7 percent of families and small deletions or insertions in 15 percent [11]. Most families have had point mutations; of families with nucleotide substitutions, less than half had mutations seen in at least one other family. All but two of these recurrent mutations occurred in CpG dinucleotides, and the mutations were spread over many of the CpG dinucleotides. The most frequent mutation was the R129H mutation in which a G to A change at the end of exon 4 replaces an arginine with a histidine. This relatively neutral substitution causes abnormal splicing and very low activity [48]. The identical mutation occurs in the *spf-ash* mouse [49]. Some correlation

of phenotype and genotype are emerging. A mutation reported [50] in the leader peptide region, which would lead to failure to enter the mitochondria, led to a severe neonatal phenotype and an absence of OTC activity in liver. Mutations at consensus splice sites caused the neonatal phenotype [44]. Overall, in patients with proven deficiency of enzyme activity, only 80 percent of mutations have been found [51].

Defective activity of the hepatic enzyme has been documented in heterozygotes, and histochemical assay of the enzyme demonstrated the presence of two populations of hepatic cells, one normal and one deficient in the activity of OTCD [52], consistent with the Lyon hypothesis. Heterozygosity has also been documented by assay of the enzyme in duodenal mucosa [53]. This method has failed to detect some known heterozygotes.

Heterozygosity has been detected by assay of the urine for orotic acid following a load of 1 g/kg of protein [23, 53, 54]. Urine is usually collected in three 4-hour aliquots following the load. More reproducible results may be obtained using an alanine load [55]. However, it is clear that the 0.7 g/kg dose that was initially employed was too large. Such a dose can lead to alarming symptoms in a heterozygote. It may also overwhelm the system in a normal and lead to a false-positive diagnosis of heterozygosity. We used 0.4 g/kg along with a very sensitive method for orotic acid using stable isotope dilution [55], and selected ion monitoring gas chromatography-mass spectrometry [56]. Both protein and alanine loading have largely been supplanted now by the allopurinol test [57]. Allopurinol inhibits the decarboxylation of orotidine monophosphate (orotidylate) (OMP) (see Figure 25.1) via reaction of its phosphorylated oxidation product on the decarboxylase. When OMP accumulates, it is reflected in the urine in the excretion of orotidine and orotic acid. The specificity of the test was reported to be greater when orotidine was measured than when orotic acid was measured [57]. If the mutation is known, it can be effectively employed in heterozygote detection, but the enormous amount of variation in OTCD often tends to make this impractical.

The ability to determine carrier status by mutational analysis (Figure 26.3) has made it clear that the allopurinol test, which was designed in families of patients with classic neonatal OTCDs, is not reliable in the families of male patients with variant phenotypes [58, 59]. In a series of 18 asymptomatic carriers, three genotypic heterozygotes were not identified by allopurinol testing [60]. In some instances, the nature of the mutation cannot be identified. In these families, linkage analysis is sometimes useful, taking advantage of restriction fragment length polymorphism. The possibility of gonadal mosaicism should be remembered in any diagnosis that a woman is not a carrier. Heterozygote detection has also been carried out for diagnostic purposes in a symptomatic girl by the ratio of transfer of administered ^{15}N-glutamine to ^{15}N-urea (^{15}N–U/G ratio) [61].

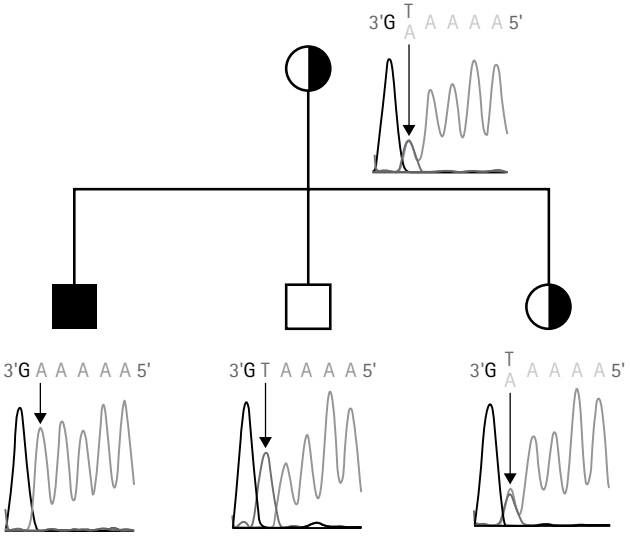

Figure 26.3 Kindred in which an A to T mutation in exon 9 of the ornithine transcarbamylase gene specified a leucine 301 phenylalanine replacement in the enzyme was identified in three individuals in two generations. The proband had a variant late onset phenotype [60]. In each of the heterozygotes, testing for orotic acid and/or orotidine after allopurinol failed to detect heterozygosity.

Prenatal diagnosis has been carried out by assay of the enzyme in the biopsied fetal liver [62]. The risk of fetal loss makes assay for the gene much more satisfactory. Prenatal diagnosis has been carried out by assay for the *TaqI* cleavage site [63], and by assay for mutations known in probands. Certainly, if precise mutation analysis is available, it should always be employed in prenatal diagnosis.

TREATMENT

The treatment of the acute hyperammonemic episode, long-term management, and the results of treatment have been set out in detail in Chapter 25. In 2012, central aspects were specified in a European guideline [19].

In the neonatal hyperammonemia of OTCD, the most vigorous intervention is required and even then, it is often impossible to avoid death or severe neurologic disability. In neonatal onset urea cycle defects, best results have been achieved in patients, especially female with OTCD, in whom prenatal diagnosis is made, but even in these patients, the risk of sudden death or disability is always present.

Most hyperammonemic infants with OTCD require hemodialysis [64]. Pharmacologic therapy with IV sodium benzoate/phenylacetate and arginine are initiated promptly and pursued vigorously. The management of intercurrent episodes of hyperammonemia in a patient rescued from the initial neonatal episode is similar, but it is hoped that treatment may be initiated promptly enough to abort the episode without the need for dialysis.

Table 26.1 Chronic management of OTCD

Citrulline/arginnine	NaPhenylbutyrate[a]	NaBenzoate	Whole protein	Essential amino acids
(g/kg per 24 hours in a child <20 kg)[a]				
0.1–0.2	≤0.25	≤0.25	0.7[b] (small infants)	0.7[b]
(g/m² per 24 hours in a child >20 kg)[a]				
2.5–6.0	5	5	[b]	[b]
(maximum doses)				
6.0 g/d	12 g/d	12 g/d	[b]	[b]

[a] NaBenzoate or NaPhenylbutyrate are given, usually not both, although some patients have been treated with both. In some patietns higher doses are needed.

[b] The FAO/WHO/UNO 2007 Report, summarized in Häberle *et al.* 2012 [17], can be used as age- and gender-dependent recommendations for energy intakes.

In OTCD, the priming infusion given over 1–2 hours contains sodium benzoate and phenylacetate 0.25 g/kg each and 0.25 g/kg of arginine-HCl (2.5 mL/kg of a 10 percent solution). The sustaining infusion given over the next 24 hours has the same content of each.

In chronic management of OTCD (Table 26.1), citrulline is substituted for arginine, as it is more palatable. Most patients are also treated with phenylbutyrate [65] as a source of phenylacetate. To many, phenylbutyrate is so unpalatable that a gastrostomy tube is required in order to avoid poor compliance, and even then, patients complain of a taste. For these reasons, sodium benzoate is preferred by some patients and some authorities. Most patients require restriction of the intake of protein, and most receive mixtures of essential amino acids to minimize the intake of nonessential nitrogen. Protein restriction and the use of benzoate/phenylbutyrate and deficiency of essential amino acids may lead to Kwashiorkor. Acrodermatitis-like skin lesions have been reported [66]. Complexities of management of OTCD were discussed in a patient diagnosed prenatally with a deletion who developed neurologic disease and anasarca, despite exemplary management and avoidance of hyperammonemia. Whole genome sequencing led to the diagnosis of a contiguous gene deletion involving chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome [10]. The authors stressed the need for detailed genetic analysis.

The prognosis in OTCD is always guarded. Prior to the development of alternate pathway therapy using ammonia scavengers (e.g. sodium benzoate, sodium phenylacetate/-butyrate), virtually all children with neonatal OTCD died in the newborn period or during infancy. Until the mid-1990s, nearly all children with early-onset type OTCD still died during the initial episode [67]. This has slowly changed with the widespread availability of ammonia measurement in hospitals, growing knowledge about the disease and the use of alternate pathway therapy. Survival rates for early-onset and late-onset OTCD patients have slowly improved but long-term morbidity is still substantial. Normal development is rare, and retardation is the most frequent outcome. In a recent meta-analysis of almost 1000 patients with OTCD, 52 percent of males presented neonatally of which 60 percent died in the initial crisis. Of the surviving infants 18 percent succumbed to subsequent decompensations until their first birthday, which only 15 percent reached without severe handicap [17]. The data for early presenting females are almost identically bleak; 43 percent die in the course of the neonatal crisis, 34 percent of the survivors consequently by their first birthday, and only 20 percent of those remained without severe handicap. These considerations have led to the use of orthotopic transplantation of the liver [61, 68, 69]. Experience has been that following transplantation, levels of ammonia are no longer a problem, and that protein restriction and medication to handle waste nitrogen are no longer required. Balancing the risks of the transplantation becomes a factor in later onset girls, but there is little question in patients with infantile presentations, that the risk of the disease is much higher. Ideally, orthotopic liver transplantation should be carried out between three to six and 12 months of age before irreversible neurologic damage has occurred, in patients with neonatal-onset disease, patients with progressive liver disease and patients suffering from recurrent severe decompensations despite intensive medical treatment [17]. A recent study, however, demonstrated that UCD children receiving a liver transplant were mainly between one to six years of age. Only 25 percent received a liver in infancy [16]. In three boys transplanted between 40 and 223 days of age [69], urea production was normal as were levels of ammonia. Two of the three performed at age-approved levels. Liver cell transplantation has now been shown in a small number of patients to improve metabolism of waste nitrogen sufficiently to buy time for an ultimate liver transplantation [70]. At removal of the recipient liver, viable islands of transplanted cells have been found.

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27

Carbamylphosphate synthetase deficiency

MAJOR PHENOTYPIC EXPRESSION

Typical neonatal hyperammonemic crisis, hyperglutaminemia, hypocitrullinemia, and absence of activity of hepatic carbamylphosphate synthetase.

INTRODUCTION

Carbamylphosphate synthetase 1 (EC 6.3.4.16) (Figure 27.1) is a mitochondrial enzyme catalyzing the formation of carbamylphosphate from ammonia in what is generally considered to be the first step in the urea cycle. It is the most abundant protein in liver mitochondria, accounting for 20 percent of the mitochondrial matrix protein [1]. Neonatal-onset patients have less than 5 percent of normal activity in the liver, whereas residual activity is higher in late-onset patients. Carbamylphosphate synthetase 1 deficiency (CPS1D) is quite rare (1:200.000–1:800.000) compared with ornithine transcarbamylase deficiency (OTCD; 1:56,000). Late-onset disease is also rarer in CPS1D. CSP1D usually presents with potentially lethal neonatal hyperammonia [2, 3]. Like many metabolic disorders, CPS1D does not arise from a common mutation; numerous mutations have been identified [4].

Transcription of the gene takes place in the nucleus. The CPS mRNA is essentially found only in the liver, but enzyme activity has been demonstrated in intestinal mucosa. Translation in the cytoplasmic ribosome yields a precursor protein, which then undergoes a complex set of molecular events that eventuate in the appearance of CPS activity in the mitochondrial matrix. The enzyme is a very large dimer of a single polypeptide with a molecular weight of 165,000,1500 amino acid residues and subunits of 160 kDa. The fusion of two domains joins subunits of striking homology in various species, even including *Escherichia coli* and yeast [5]. An amino terminal leader sequence targets the protein to the mitochondria and is highly basic because of its content of lysine and arginine residues. Following transport to the mitochondria, this sequence is cleaved to yield the mature protein [6–8]. A defect at any stage of the sequence from transcription and translocation to transport, uptake, and processing could lead to loss of enzyme activity and clinical disease.

The human cDNA has been cloned and mapped to chromosome 2q35 [9, 10]. Mutations reported have included T1370G, and A2429G, which led to V457G and Q810R amino acid substitutions [10]. Molecular analysis revealed no immunoreactive enzyme and no translatable mRNA in some patients with lethal neonatal disease [11]. In 2011, Häberle *et al.* reported 192 different pathogenic mutations in the CPS1 gene, including 130 novel mutations [4]. When combined with previous reports, it is clear that most mutations (90%) are private. The few recurrent mutations tended to occur at CpG dinucleotides.

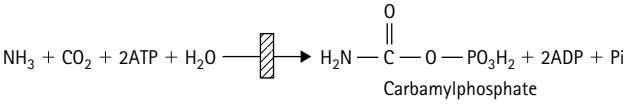

Figure 27.1 The carbamylphosphate synthetase reaction. Acetylglutamate is an obligate activator of the enzyme.

CLINICAL ABNORMALITIES

The clinical abnormalities of CPS1D are indistinguishable from those of OTCD (Chapter 26). Usually, the infant is normal at birth and may do well for a short period, often until feedings begin. Then failure to feed well

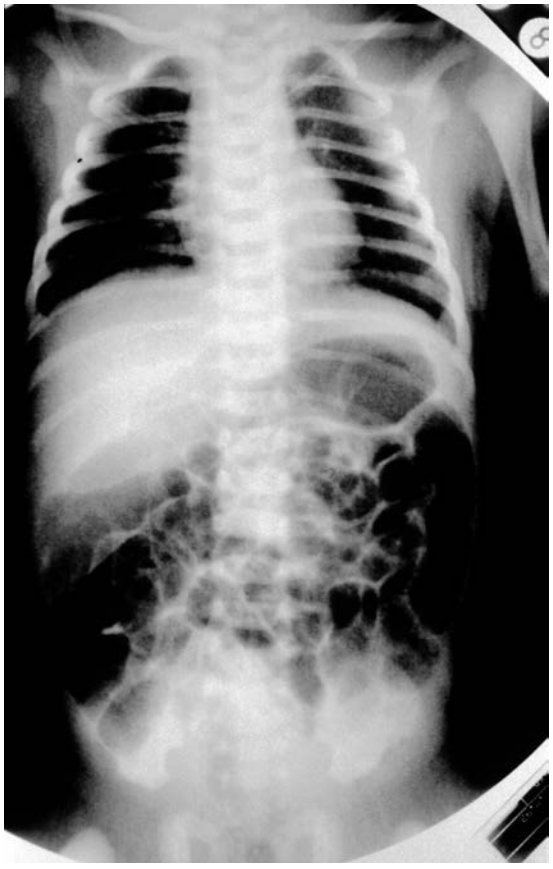

Figure 27.2 Abdominal radiogram of a child with CPS1D at day 3. Massively dilated intestines, especially the colon ascendens and transversum, mimicking primary abdominal disease.

and lethargy develop. There may be grunting or rapid respiration, hypotonia or hypertonia, convulsions, and hypothermia. Sometimes initial abdominal symptoms (vomiting, disturbed transport) may be so prominent that abdominal disease is suspected and investigated (Figure 27.2) [12]. Symptoms are rapidly progressive to deep coma, in which there is a complete unresponsiveness to stimuli. We have compared this state to surgical anesthesia. Apnea supervenes and the infant survives only with assisted ventilation. The history often reveals that siblings died very early in life. Compared to distal urea cycle disorders (argininosuccinate synthetase deficiency and argininosuccinate lyase deficiency; see Figure 25.1), subjects with CPS1D and OTCD present earlier – mostly between 24 and 72 hours of age – and with a higher initial peak-blood ammonia level (PBAL) [13].

A typical history was that of an infant who began to feed poorly, became lethargic, and had convulsions and an abnormal electroencephalogram [14]. She was admitted to hospital at 20 days. Two of her siblings had died with similar symptoms including persistent vomiting at around four weeks of age. Despite treatment with a low protein diet, she also died at seven months [14, 15]. Another full-term infant appeared normal at birth and was nursed at 10 hours; at 24 hours, he developed profuse sweating and nursed poorly

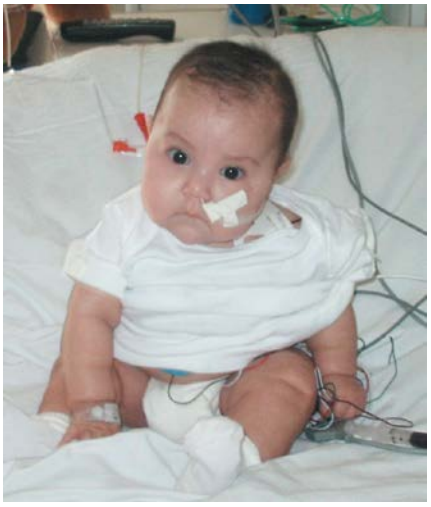

Figure 27.3 AO: An eight-month-old infant with CPS1D. The picture was taken following recovery from hyperammonemic coma and just prior to a successful liver transplantation.

[2]. He had irritability, hypothermia, and hypertonia, along with opisthotonus and ankle clonus. He developed coma and died at 75 hours of age. Of 20 patients reported with this neonatal presentation (outcome was known in 17), all but six died in the neonatal period, and the exceptions died at 5–15 months [2]. An infant with this type of disease is shown in Figure 27.3. Her brother, now mentally impaired, preceded her so she was spared the initial neonatal episode, but she had almost monthly admissions to hospital for hyperammonemia. This clinical picture is seen in the other urea cycle disorders, which present in the neonatal period. It can be distinguished from the hyperammonemia that occurs in organic acidemias because the serum concentrations of electrolytes do not reveal an acidosis. Blood gases more often indicate a respiratory alkalosis.

A very different type of disorder, the partial deficiency of carbamylphosphate synthetase, was exemplified by a 13-year-old girl [16]. She had episodes of vomiting and lethargy at three weeks and 13 months of age, and she had a transient hemiparesis at two years. She had spastic quadriparesis and severely impaired mental development. A similar patient [17] presented at nine years of age with hyperammonemic coma and was thought to have Reye syndrome. She recovered, but with extensive damage to the brain. She had seizures at seven days and slow psychomotor development, but was a below average student in a regular school before the episode at nine years. From six years, she had episodic vomiting, abdominal pain, and muscle weakness, lasting 2–3 days. Other patients have had intermittent vomiting and screaming episodes or intermittent lethargy. Impaired mental development has been the rule in those with childhood onset. Seizures and cerebral atrophy on imaging scans are common. We have seen an adult with CPS1D who developed his first episode of hyperammonemic coma at the age of 36 years (Figure 27.4). This followed a fugue-like episode for which he had

Figure 27.4 CB: A 38-year old man with CPS1D.

no memory. He gave a history of two previous episodes in which he had periods of a few days for which he had no memory and unexplained scrapes and bruises.

Metabolic stroke has been reported in CPS1D [18]. An 18-month-old girl was admitted with somnolence and a left hemiparesis. Magnetic resonance imaging (MRI) revealed infarction of the area supplied by the right middle cerebral artery, but carotid angiography revealed no obstruction of this vessel.

Presentation with coma following the use of valproate to treat seizures was reported in a 32-year-old woman [19]. Onset of agitated disorientation followed by coma and decerebrate posturing and death was reported [20] in a previously asymptomatic woman following pregnancy and delivery.

The clinical chemistry of CPS1D may be unremarkable except for the hyperammonemia, or there may be respiratory alkalosis. Amino acid analysis of the plasma at the time of hyperammonemia reveals elevation in glutamine and usually alanine, and sometimes aspartic acid. In addition, the concentration of lysine may be elevated. The concentrations of arginine and citrulline are typically low, but values within the normal range have been encountered especially in late-onset disease. Organic acid analysis of the urine is remarkable for the absence of elevations of either orotic acid or uracil. The excretion of 3-methylglutaconic acid may be quite high. Carnitine deficiency has been reported in CPS1D [21].

GENETICS AND PATHOGENESIS

CPS1D is determined by mutation in an autosomal recessive gene.

The molecular defect is in the mitochondrial carbamylphosphate synthetase (EC 6.3.4.16) (see Figure 27.1). There are two distinct carbamylphosphate synthetases [22]. The one found exclusively in the cytosol, which has been designated CPS2, is involved in pyrimidine biosynthesis. This enzyme is particularly active in rapidly growing tissues and preferentially utilizes glutamine rather than $NH4^{+}$, and it is not acetylglutamate dependent. The ammonia-dependent mitochondrial carbamylphosphate synthetase has been designated CPS1. The two CPS enzymes are immunologically distinct. In the mitochondria, CPS1 catalyzes the formation of carbamylphosphate from NH_4^{+}, HCO_3^{-} and 2 ATP. Glutamine is not an effective substrate, and acetylglutamate and Mg^{++} are required. X-ray crystallography showed that each ATP is bound at a separate fold in the CPS molecule in the catalysis of the carbamylphosphate product [23]. The two CPS enzymes are located in distinct cellular compartments, but carbamylphosphate, which accumulates in mitochondria when there is a defect in OTC or more distal enzymes, readily makes its way to the cytosol and becomes a substrate for the synthesis of pyrimidines [24], and orotic acid is found in the urine. This is, of course, absent in CPS1D. The concentration of urea may be low. Concentrations of citrulline and arginine may be quite low.

In addition to acetylglutamate, carbamylglutamate is an activator of CPS1; glutamate and 2-oxoglutarate are inhibitors [25], as is carbamylphosphate, the product [26].

Deficiency of CPS is readily demonstrable by assay of the enzyme in the biopsied liver [2, 27]. It is possible to make the diagnosis of CPS1D by assay of the enzyme in biopsied duodenal [27] or rectal [28] tissue. In patients with CPS1D, levels of 0–50 percent of normal have been reported [14–16, 28]. In general, the correlation of residual activity with clinical presentation has been quite good, but it is also sometimes lacking. Klaus *et al.* (2009) reported a man who presented at 45 years with episodes of confusion and bizarre behavior [29]. He was initially thought to have some form of epileptic encephalopathy, until an elevated ammonia level led to the diagnosis of CPS1D. His grandson presented on day two with neonatal onset coma and finally died at three years of age. Assay of CPS1 activity can be faster in confirmation of CPS1D than molecular genetics, but it requires invasive techniques to gain biologic tissue and is only available in very few research laboratories worldwide [30].

Heterozygosity has been documented in a family in which intermediate levels of CPS1 activity were documented in the biopsied liver of parents [31]. Antenatal diagnosis is possible by assay of the enzyme in biopsied fetal liver or by mutational analysis. In the few families with incomplete or inconclusive mutation status, the frequent RFLP at the CPS1 locus found after incubating with BgI1 is useful in heterozygote detection and prenatal diagnosis. Linkage dysequilibrium among four restriction patterns has been found; the A pattern was found in a frequency of 0.83 in affected individuals and 0.20 in controls [32].

The gene (*CPSI*) is enormous. It contains 38 exons [33]. It is located on chromosome 2q35 and spans 122 kb. Most mutations identified have been missense [4, 10], which led to markedly reduced enzyme activity. In 16 Japanese patients, 25 mutations were identified, 19 of them novel [34]. Mutations common to more than one family were found in 32 percent. Missense mutations clustered around the phosphorylation domains. Nonsense and in/del mutations were widely scattered. In this series, most patients presented in the neonatal period, but a later onset did not ensure a favorable prognosis. One patient died on presentation at 31 years.

A C+A polymorphism in the gene (p.T1405N) altered the production of nitric oxide, and vascular muscle reactivity and blood flow was greatest in those homozygous for the allele [35].

TREATMENT

The treatment of the acute hyperammonemic crisis of CPS1D is as outlined in Chapters 25 and 26 [30, 36, 37]. The acute and chronic management of this condition does not differ from that of OTCD (see Table 26.1). Doses of Na-benzoate and Na-phenylacetate begin with 250 mg/kg of each. Arginine is given at 250–500 mg/kg/day during acute crises and with 100–200 mg/kg/day long term. For oral treatment, citrulline is a good alternative. Adjustments are done according to the results of amino acid determinations ensuring high normal values. An antiemetic such as zofran (0.15–0.5 mg/kg) is sometimes useful. For chronic oral treatment, Na-phenylacetate has been employed in doses of 250 mg/kg per day. The dietary intake of protein is restricted and supplementation with a mixture of essential amino acids (cyclinex, EAMI, UCD) is usually helpful in maintaining reasonable concentrations of amino acids in plasma while minimizing nitrogen load.

N-carbamylglutamate is an analog of n-acetylglutamate which activates carbamylphosphate synthetase [38]. The advantage is that it enters mitochondria and is not hydrolyzed by cytosolic deacylases, as is acetylglutamate. The compound, at 100–300 mg/kg has been reported to be effective in the management of acetylglutamate synthetase (AGS) deficiency [39, 40]. Doses of 300–1800 mg/day have been well tolerated, and long-term management has been reported. The compound had been suggested [40] as a test for AGS deficiency in acute hyperammonemic crisis of unknown course, which might distinguish it from CPS1D. However, the compound has now also been reported to be quite effective in the management of single patients with CPS1D [41].

Prior to the development of alternate pathway therapy using ammonia scavengers (e.g. sodium benzoate, sodium phenylacetate/-butyrate), virtually all children with neonatal CPS1D died in the newborn period or during infancy. Between the 1980s and mid-1990s, the one-year survival rate for children with early-onset type UCD was approximately 50 percent and worse for early-onset CPS1D [42]. This, however, has changed with the widespread availability of measurement of ammonia in hospitals, growing knowledge about the disease and the use of alternate pathway therapy. From approximately 150 patients with CPS1D assembled between 1980 and report, the frequency of neonatal crises is >70%. In a monocentric study from France, the proportion of CPS1D patients presenting with neonatal crises was even 93 percent [43]. Survival of the neonatal period after early onset acute hyperammonemic crisis is now 68 percent, but of those, 34 percent still die in infancy and 47 percent are severely handicapped. These data were confirmed by a recent study including 103 subjects with neonatal-onset UCDs [13].

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28

Citrullinemia type I

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal coma, convulsions, hyperammonemia, hypercitrullinemia, orotic aciduria, and defective activity of argininosuccinate synthetase.

INTRODUCTION

Citrullinemia was first reported in 1962 [1] in a patient with impaired mental development. Soon after, it became apparent that the classic presentation is as a typical neonatal hyperammonemia that was, until the development of modern methods of pharmacologic therapy, uniformly lethal [2–8]. The picture is indistinguishable from that of the male neonate with ornithine transcarbamylase deficiency (Chapter 26). The activity of argininosuccinate synthetase (EC 6.3.4.5) is widely expressed in tissues (Figure 28.1). Its deficiency is readily demonstrated in cultured fibroblasts [9]. The gene (*CTNL1*) has been cloned [10] and mapped to chromosome 9 at q34.11 [11]. About 100 mutations have been described [12–15].

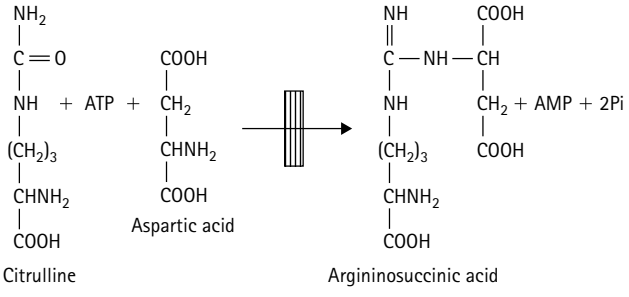

Figure 28.1 The argininosuccinic acid synthetase reaction, site of the defect in citrullinemia.

CLINICAL ABNORMALITIES

Citrullinemia usually presents as an overwhelming neonatal illness. Following a brief hiatus of 72 hours to one week in which the newborn appears normal, anorexia, vomiting, and lethargy develop, and these symptoms are followed rapidly by progression to deep coma (Figures 28.2–28.7). Apnea ensues and death is inevitable, unless the infant is intubated and provided with mechanical ventilation. Seizures often occur and there are abnormalities of the electroencephalogram (EEG). The infant may have hypertonia and there may be decerebrate posturing. Neurologic findings usually include increased deep tendon reflexes and papilledema [6]. The neurologic abnormality is progressive to flaccidity and dilated, fixed pupils. The infant is unresponsive even to deep pain. The liver may be enlarged and serum levels of transminases are often elevated.

Citrullinemia is genetically heterogeneous and there has been a variety of different clinical pictures in patients with partial residual activity of the defective enzyme. All of these variants are encountered less frequently than the classic neonatal one, often associated with weaning or switching from formula to cow's milk or intercurrent illnesses. Some of these patients have had a more gradual onset of difficulty with feedings and recurrent or cyclic vomiting in infancy. Some have had hepatomegaly and elevation of the serum glutamate-oxaloacetate transaminase (SGOT) or serum glutamate pyruvate transaminase (SGPT), which may cause confusion by suggesting a diagnosis of hepatocellular disease [6]. This is also true of the classic acute infantile

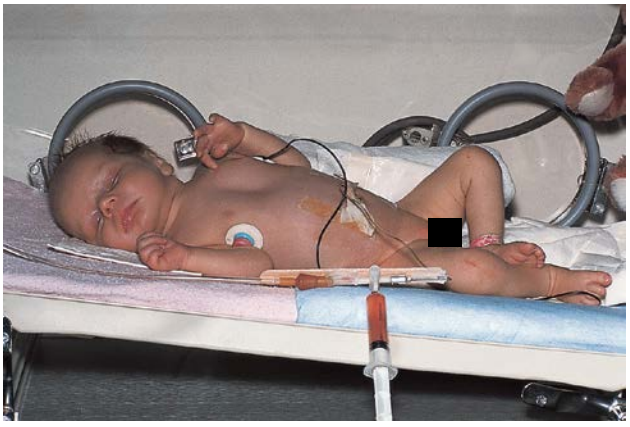

Figure 28.2 JPN: A 12-day-old infant with citrullinemia, illustrating deep coma requiring assisted ventilation. The concentration of ammonia in plasma was 770 μmol/L. He was flaccid and completely unresponsive. He had required assisted ventilation, but this was discontinued after a series of exchange transfusions that temporarily lowered the ammonia to 236 μmol/L. Concentrations of ammonia were over 1000 mg/dL at four hours.

disease [16]. The prothrombin and partial thromboplastin time may be prolonged. Cirrhosis at 17 months of age has been reported [17]. Acute hepatic failure which led to referral for liver transplantation occurred in two infants [18] in whom medical treatment reversed the hepatic changes. Episodic hyperammonemia may occur with vomiting, anorexia, lethargy, headaches, tremors, irritability, behavioral changes, seizures, or ataxia often progressing to stupor or coma [19]. Intercurrent hyperammonemic episodes in

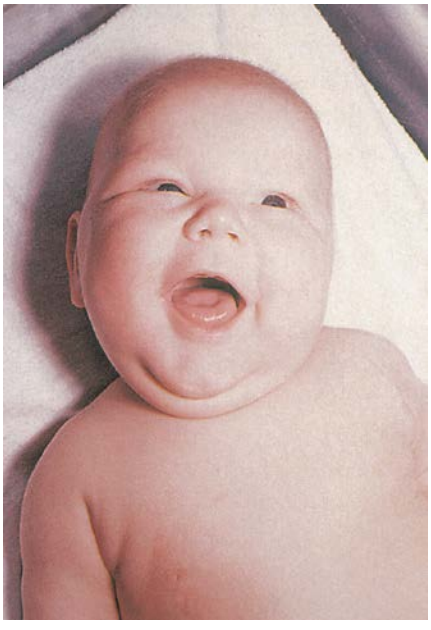

Figure 28.3 JPN: Two months after the time of Figure 28.2. Treated with keto acid analogs of amino acids, he had recovered well and was alert and appeared to be developing normally. He was rather chubby. He died before his first birthday of acute hyperammonemic coma.

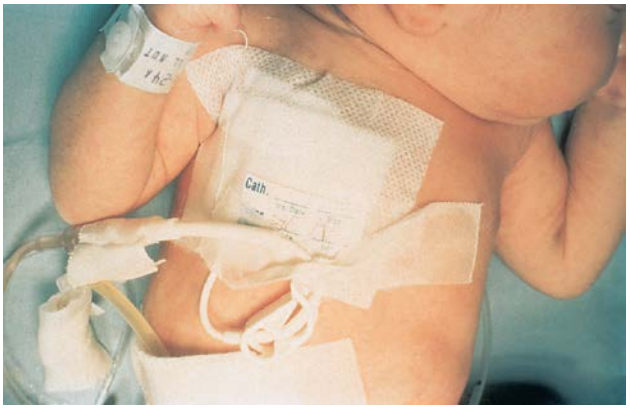

Figure 28.4 RM: A newborn with citrullinemia. He developed hyperammonemic coma at three days of age. He was treated with exchange transfusion, arginine, and Na-benzoate/phenylacetate. The hyperammonemia resolved, but the level of citrulline in plasma was more than 1000 μmol/L.

affected individuals have been precipitated by high-protein meals, infections, seizures, medication (especially steroids, valproate, haloperidol and L-asparaginase/pegaspargase), menstruation, severe exercise, trauma or burns, and surgery. The postpartum period (due to catabolism and the involution of the uterus) is an important trigger for late-onset hyperammonemia [20–22]. Some degree of impaired mental development is usually present [1], and computed tomography (CT) or magnetic resonance imaging (MRI) scan usually reveals evidence of cerebral atrophy.

One patient in whom citrullinemia was found by family investigations had had no clinical evidence of disease at the time of report [23]. In countries in whom extended newborn screening programs have included the determination of citrulline, very mild variants are now being increasingly detected, which mostly remain completely free of symptoms, and do not need constant treatment [25]. However, they may still be at risk for metabolic crisis. Even the patient with a variant form of citrullinemia, in whom symptomatology

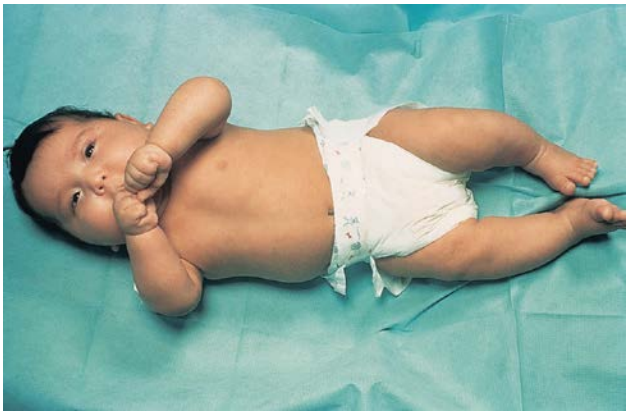

Figure 28.5 RM: Four months later. He was rather obese and had frequent diaper rashes consistent with protein inadequacy. In follow up, he had mildly impaired mental development despite having had only two subsequent slightly hyperammonemic attacks.

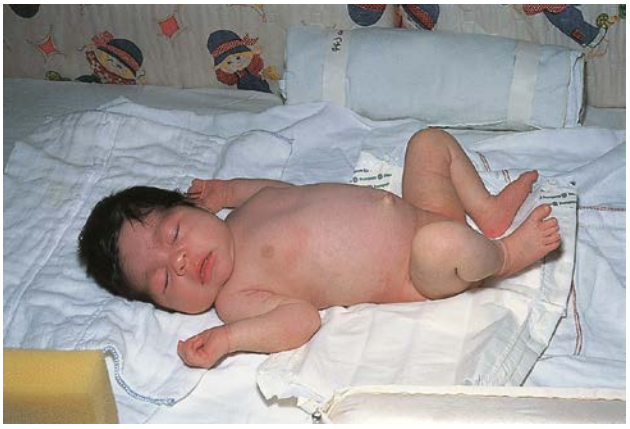

Figure 28.6 NF: At four days of age. She appeared to be normal and had a normal level of ammonia, EEG, and CT scan. Treatment was initiated at birth. Her sister had died of citrullinemia in the neonatal period. A prenatal diagnosis permitted treatment of NF from birth, which prevented neonatal hyperammonemia. The concentration of citrulline in the amniotic fluid at mid-gestation was 80 μmol/L (1.4 g/dL) (normal range, 0–23 mmol/L) and the activity of the enzyme was eight percent of the control mean. She had many other episodes of acute hyperammonemia, and they seemed to become more frequent and more worrisome during teenage years. A liver transplantation at the age of 16 years led to a cessation of these episodes.

has been mild, or even absent for long periods, may become neurologically incapacitated in childhood or adulthood and should be followed. Patients with this disease have had strokes (Appendix) [24].

The most prominent metabolic characteristic of citrullinemia is the hyperammonemia, which is usually massive in the neonatal form, but deep coma, mimicking anesthesia, has been seen with concentrations of 400 mmol/L [16]. Concentrations in the blood of glutamine and usually of alanine are also elevated, and often that of aspartic acid as well. The concentration of arginine is usually decreased. Excretion of orotic acid is increased [21], although not usually to the degree seen in ornithine transcarbamylase deficiency.

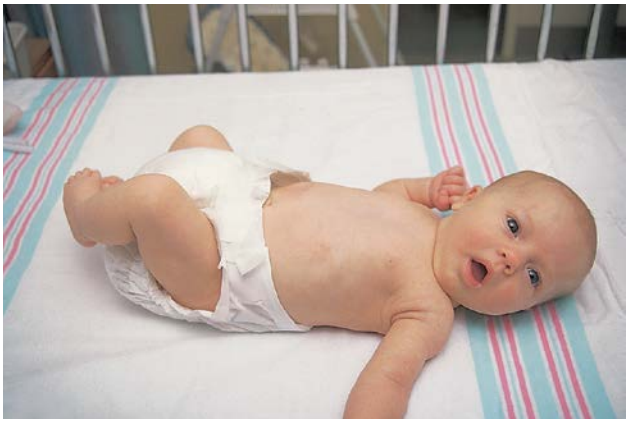

Figure 28.7 VT: An infant with citrullinemia who appeared normal at two months. A previous sibling had died of overwhelming neonatal citrullinemia.

Concentrations of citrulline in body fluids of these patients are very high. Plasma concentrations usually approximate 40–100 times normal; levels of 850–4600 μmol/L are commonly encountered. The lowest level reported of 290 μmol/L is found in patients who had no clinical evidence of disease [25]. Urinary excretion of citrulline may range from several hundred milligrams per day in an infant to several grams per day in an older patient. Some patients may also excrete homocitrulline, homoarginine, or N-acetylcitrulline [1, 2, 6, 18, 19, 26]. Concentrations of citrulline are also elevated in the cerebrospinal fluid (CSF), but levels are lower than in the blood.

There is another monogenic metabolic disease which leads to elevated levels of citrulline, caused by deficiency of the amino acid transporter citrin (also called citrullinemia type 2). It is common in East Asians and usually presents in adults with hyperammonemia and neuropsychiatric disease. It may also cause neonatal/infantile cholestatic liver disease without hyperammonemia which is mostly transient. In adult-onset citrin deficiency, plasma ammonium concentrations are "only" about 5- to 10-fold elevated during acute episodes, and citrulline concentrations are approximately elevated 20-fold. Interestingly, arginine concentrations are normal to mildly increased rather than decreased due to the fact that argininosuccinate synthetase is also expressed in the small intestine and the kidney. These two organs are the main sources of arginine synthesis and are not affected in citrin deficiency.

GENETICS AND PATHOGENESIS

Citrullinemia is transmitted as an autosomal recessive disease. Intermediate levels of activity of argininosuccinate synthetase have been found in fibroblasts of parents [7–9, 16]. Molecular genetic analysis is the preferred prenatal testing method [21]. Prenatal diagnosis has also been made by assay of the concentration of citrulline in amniotic fluid or by assay of the enzyme in cultured amniocytes (see Figure 28.6) [4, 16]. Prenatal diagnosis may also be accomplished by assay of the enzyme in chorionic villus material [27], but very low levels of enzyme in heterozygotes may be a real problem. For this reason, a sensitive radiochemical assay was developed. The first successful live birth following preimplantation genetic diagnosis for citrullinemia was reported in a Korean couple heterozygous for ASS1 mutations [28].

The molecular defect in citrullinemia is in the enzyme, argininosuccinic acid synthetase. This is a cytosolic enzyme in contrast to ornithine transcarbamylase and carbamylphosphate synthetase (Chapters 26 and 27). Argininosuccinic acid synthetase catalyzes the conversion of citrulline and aspartic acid to argininosuccinic acid (see Figure 28.1). The enzyme is widely distributed in tissues. The defect has usually been demonstrated in cultured fibroblasts [8, 9], and it has also been demonstrated in the liver [1, 4, 19]. Neonatal-onset cases have less than five percent of normal activity in liver, whereas late-onset cases have 10 to 25 percent of normal activity [29].

Kinetic studies have revealed Km values for citrulline as high as 200 times normal in variants with alterations in the structure of the enzyme protein [9, 30]. In one patient, the activity of the enzyme in brain was lower than that found in the liver [31].

Cloning of the cDNA for argininosuccinic acid synthetase was facilitated by the use of a cultured human cell line in which very high levels of mRNA for this enzyme were produced when the cells were cultivated in medium containing canavanine, an analog of arginine [10]. The gene was sequenced and found to contain 63 kb in 16 exons [11]. It codes for a monomeric protein of 46 kDa that forms a tetramer [32]. Expression of the gene is highly regulated, increasing with fasting, dexamethasone, or dibutyryl-cAMP, and the substitution of citrulline for arginine in medium, and decreasing in response to arginine.

Analysis of the DNA of 11 patients by Southern blot failed to reveal major rearrangements of the gene [33]. Analysis of the mRNA and protein of these lines revealed considerable heterogeneity. Nine of 11 were devoid of cross-reacting material (CRM), while they had all mRNA. Levels of CRM and enzyme activity below 50 percent were found in some parents. Among patients with classic citrullinemia close to 100 mutations have by now been reported [13, 34], indicating a considerable heterogeneity, but with most mutations in exons 15, 12, 13, and 14. Most patients are compound heterozygotes. A frequent mutation has only been identified in Japan, where 10 of 23 affected alleles had the same mutation, deletion of exon 7 (IVS6-2A-G) [34]. This differed from the situation in Europe or the United States, where far greater heterogeneity of mutations has been found with G390R being the most frequent in patients with the classic phenotype [35].

Heterozygosity has been discussed to be responsible for mild phenotypic presentations such as now detected by newborn screening. It has been demonstrated that mild citrullinemia can be caused by homozygous mutations (p.Trp179Arg and p.Gly362Val) in the argininosuccinate synthetase gene [35]. Recently, a multicenter study from Spain yielded two new mutations (p.Arg86Cys and pThr91Ala) and suggested that they could also be related to good prognosis of CTLN1 [36].

An interesting homozygous mutation found in the child of consanguineous parents was a G-to-C substitution in the splice acceptor site of the terminal intron, which abolished normal splicing and led to three abnormal splice products, the most common of which was translated to a protein 25 amino acids longer, but so unstable that CRM was not detectable [12]. Most of the other alterations found have been missense mutations (Table 28.1) [13, 34]. A number have involved CpG dinucleotides. Deletions of entire exons have resulted from deletion of genomic sequences [14]. Nonsense mutations were until recently not reported, but a nonsense mutation in codon 86 (arginine) was found in citrullinemic cattle [15]. The missense mutations found in classic neonatal citrullinemia have all altered an amino acid that was highly conserved, most of them across eight species ranging from humans to *Saccharomyces* and *Escherichia coli* [34]. Elucidation of the genomic sequence of the gene has permitted the use of intronic primers and has simplified mutational analysis [35]. In a patient in whom no mRNA could be detected, a transposition at 279 converted an arginine to a stop codon [37]. It was concluded that the RNA negative phenotype resulted from nonsense-mediated mRNA decay.

Table 28.1 Mutations defined in classic citrullinemia

Mutation	Deletions, insertions	Exon
G14S		3
S18L		
		4
R86C	Del Exon 5	5
A118T		
	Del Exon 6	6
	IVS6-2	
R157H	Del Exon 7	7
S180N		8
A192V		9
		10
		11
R272P		12
R279Q		
G280R		
R304W	Del Exon 13	13
G324S		
R363W		14
R363L	IVS15-1	
G390R	Insertion 37 b, exons 15, 16	15
	Del 76 Exon 16	16

Abbreviation: b, bases; Del, deletion; A, alanine; C, cysteine; G, glycine; H, histidine; L, leucine; N, asparagine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan.

TREATMENT

The acute management of the initial neonatal hyperammonemia and subsequent intercurrent attacks is set out in Chapter 25, along with general principles of long-term management of hyperammonemic infants using sodium benzoate and/or phenylacetate and arginine. The infant with citrullinemia differs in that even an acute crisis of hyperammonemia can often be managed with IV arginine alone, as long as the episode is treated promptly and the level of ammonia is not too high. Of course, citrulline should not be employed. It would not be recommended to treat the initial infantile crisis with anything less than a full-scale attack on the hyperammonemia. In this disease, whether benzoate and/or phenylacetate is employed, it is well to employ doses of arginine in both the priming and sustaining infusion of at least 0.66 g/kg. In the event that ammonium concentrations do not respond to this management and biochemical or clinical symptoms worsen,

continuous veno-venous hemodiafiltration should be started immediately (planned and organized earlier, i.e. the latest at levels >400 μmol/L) in neonates or children with ammonia levels of > 500 μmol/L or at lower levels if response to medical treatment is inadequate.

Long-term steady-state management can usually be provided with arginine and a diet (Figure 28.8) [16, 38]. In many children, a modestly protein restricted diet is sufficient, but the use of a high-caloric, low-protein diet supplemented with essential amino acids and, if necessary, vitamins and minerals may be necessary. The principle is that if ornithine molecules can be provided through supplemental arginine, in order to keep the urea cycle operating, waste nitrogen can be adequately eliminated in the form of citrulline. Citrulline contains only one more nitrogen atom than the two provided as ornithine. It is probably not as efficient as urea in eliminating nitrogen, but this is sufficient except at times of catabolism. If the patient is vomiting or for some other reason cannot take oral arginine, hospital admission for IV arginine therapy is mandatory. Oral doses of arginine employed have ranged from 0.25 to 0.8 g/kg per day. Normal development has been reported with such a regimen [38]. We have observed low levels of essential amino acids, especially of branched amino acids, in patients treated with benzoate, phenylacetate, or phenylbutyrate. This has also been reported [39]. There may be clinical signs of protein inadequacy (see Figures 28.5 and 28.9). Supplementation with mixtures of essential amino acids is preferable to increasing whole protein in this instance, and nitrogen-free analogs of amino acids are useful [40], but these mixtures are no longer available. This problem of essential amino acid depletion has not been observed with arginine treatment.

Prognosis for intellectual development depends on the nature of the initial hyperammonemia, especially its duration [41] or those of recurrent episodes. The ability to prevent hyperammonemia by early treatment in patients diagnosed prenatally [42] should be consistent with a better prognosis, but only if recurrent attacks of hyperammonemia are prevented or treated early and effectively. The highest survival rates were reported for Japan with one-year survival rates for early-onset and late-onset corresponding to 90 percent, or even 100 percent, respectively [20]. A recent study demonstrated that early-onset disease manifestation, a peak-blood ammonia level >1000 μmol/L and coma on admission are associated with the highest risk of mortality during a hyperammonemic episode [43]. In a recent meta-analysis of 300 patients with citrullinemia, 65 percent presented neonatally, of which 67 percent survived. Of the surviving infants, 20 percent succumbed

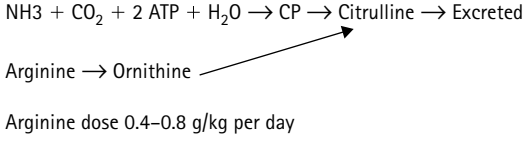

Figure 28.8 Chronic management of citrullinemia. Of the three N atoms of citrulline, two come from ornithine administered as arginine. Hence, a net of one N is lost for every molecule of citrulline excreted. As long as ornithine is supplied, this minicycle continues to operate. CP, carbamylphosphate.

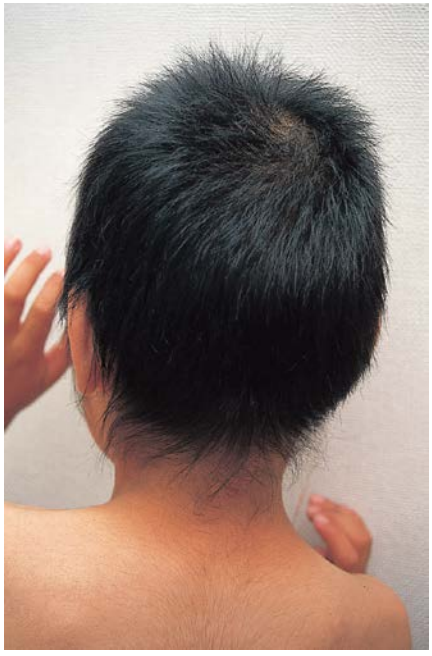

Figure 28.9 NF: Her very short hair illustrates the fact that among patients with urea cycle defects, the loss of hair has been our most sensitive index of protein inadequacy.

to subsequent decompensations before their first birthday, which only 36 percent reached without severe handicap [44]. Therefore, the possibility of a liver transplantation should be pursued in patients with severe neonatal-onset disease and patients suffering from severe decompensations despite intensive medical treatment [21]. After transplantation, hyperammonemia is controlled but citrulline concentrations remain elevated and arginine concentrations remain low. Thus, long-term arginine supplements may still be required following liver transplantation [45].

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29

Argininosuccinic aciduria

MAJOR PHENOTYPIC EXPRESSION

Hyperammonemia leading to lethargy and coma; convulsions; hepatic fibrosis, hypertension; hyperglutaminemia; argininosuccinic aciduria and acidemia and defective activity of argininosuccinate lyase.

INTRODUCTION

Argininosuccinic aciduria was described in 1958 as the first defect of the urea cycle [1]. The first patients recognized had chronic, more indolent disease where the major manifestations were nonspecific, sometimes mild or moderate mental impairment [2–4]. This may reflect the unique features of the hair in this disorder, which brought many of the early patients to attention with apparent alopecia. The disorder also presents, and as now recognized more frequently, in the classic neonatal hyperammonemic pattern of a typical urea cycle disease [5–10]. Sometimes these infants may be suspected clinically to be different from those with other urea cycle disorders because of the magnitude of the hepatomegaly.

The enzyme argininosuccinate lyase, or argininosuccinase (EC 4.3.2.1) (Figure 29.1), catalyzes the conversion of the argininosuccinate formed from citrulline and aspartate, to fumarate and arginine, the last compound of the urea cycle prior to the urea splitting off. The cDNA for the human gene has been cloned [11] and the gene has been localized to chromosome 7q11.21 [12]. Mutations have been defined [13–15], some of which have led to alternative splicing.

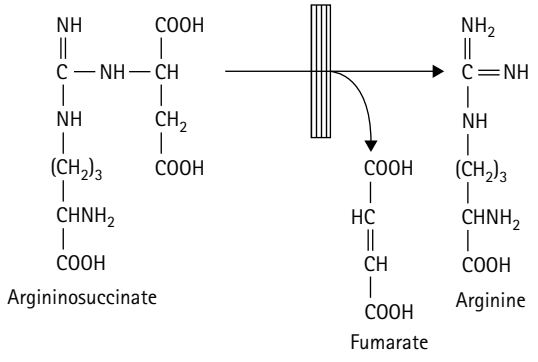

Figure 29.1 The reaction catalyzed by argininosuccinase.

CLINICAL ABNORMALITIES

The classic presentation of argininosuccinic aciduria, in 60 per cent of all patients, is as overwhelming illness in the newborn period [7]. Prior to the development of modern methods of pharmacologic therapy, the end result of this presentation was uniformly fatal [16]. The picture is indistinguishable from that of the male infant with ornithine transcarbamylase deficiency (Chapter 26). Following a brief hiatus, in which the newborn appears normal, anorexia or vomiting and lethargy develop, and these symptoms are rapidly progressive to deep coma, apnea, and death, unless the baby is intubated and maintained via mechanical ventilation. Seizures often occur. Abnormalities of the electroencephalogram (EEG) consist mainly of multiareal spikes, spike-waves, or sharp-and-slow-wave activity. There may also be sustained monorhythmic theta activity [17]. The infant may have hypertonia or hypotonia or there may be decerebrate posturing. This condition is progressive to flaccidity and dilated fixed pupils. The infant is unresponsive even to deep pain. There may be hypothermia. Patients may have tachypnea and respiratory alkalosis, which are general consequences of hyperammonemia [18]. A bulging fontanel usually indicates the presence of cerebral edema, but

cerebral hemorrhages have been seen in hyperammonemic infants, as have fatal pulmonary hemorrhages.

As in the case of other disorders of the urea cycle (Chapter 26 [ornithine transcarbamylase deficiency], Chapter 27 [carbamylphosphate synthetase deficiency], Chapter 28 [citrullinemia]), argininosuccinic aciduria is genetically heterogeneous and patients with variant forms of the enzyme, in which there is partial residual activity, may have more indolent forms of the disease [19]. Such patients may present simply with impaired mental development [3, 20] or a convulsive disorder. Commonly, there is episodic disease, such as cyclic vomiting or recurrent headache, ataxia, tremulousness, or lethargy. The classic, and most common phenotype is the neonatal form, with rapid progression to coma in the first days of life. In a subacute or late-onset type, the disease becomes manifest in late infancy or childhood [7, 20]. They may survive with impaired mental development, or seizures. Movement disorders are prominent in about one third of patients [21]. Some have trichorrhexis nodosa. One patient reported at 30 years [19], presented at ten years of age with intention tremor. There was no hyperammonemia or encephalopathy. He was described as having mildly impaired mental development, but had attended regular school, could read, write, drive a car, work in a factory, and father a son.

Abnormalities of the EEG are common [17]. Cerebral atrophy may be evident on computed tomography (CT) or magnetic resonance imaging (MRI). Others may have episodes of hyperammonemic encephalopathy and coma thought to be Reye syndrome or encephalitis. Hyperammonemia is often precipitated by infection and such an episode may be fatal.

A unique finding in patients with variant forms of argininosuccinic aciduria is trichorrhexis nodosa (Figures 29.2–29.7) [2, 3, 22]. These patients may appear hairless at a distance, but there is always at least a fuzz of short hair. More often, they have short dry hair, but never need a haircut. The hair is very friable and breaks off easily. There may be a history of hair on the pillow. Under the microscope, the hair sheaths contain tiny nodules (Figure 29.8). Break points may be seen at the nodules.

In addition, about half of these patients have hepatomegaly [21, 23]. Serum values of the transaminases are elevated at least at times of hyperammonemia [21, 23, 24]. Progressive hepatic fibrosis has been diagnosed and there may be ultrastructural abnormalities in hepatocytes. Synthetic functions are usually normal. Chronic coagulopathy was reported in an eight-year-old (Figures 29.6 and 29.7) with prolongation of prothrombin time and increase of the partial thromboplastin time (PTT); the only clinical consequence in the patient reported was prolonged bleeding at venepuncture sites [24].

More neonatal patients with argininosuccinic aciduria survive acute hyperammonaemic crisis than in other urea cycle disorders (81%) [7], but they are likely to be left with impaired mental development. Some have developed spasticity, ataxia or a seizure disorder.

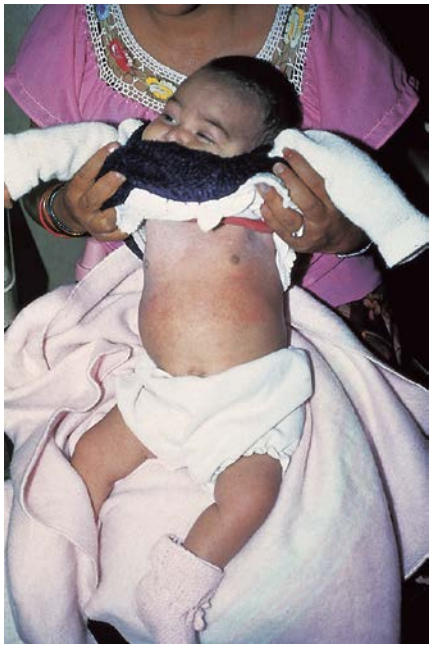

Figure 29.2 CGG: A Mexican infant with argininosuccinic aciduria. The dermatitis on the abdomen and chest was unrelated.

Figure 29.3 CGG: Her hair was short and brittle.

Hypertension has been observed in patients with argininosuccinic aciduria. This has been thought to relate to nitric oxide synthesis [21, 25]. Citrulline and arginine are the precursor of nitric oxide, which is relevant to smooth muscle arteriolar function.

Argininosuccinic aciduria has been reported in patients, detected by routine neonatal screening, and treated with protein restriction and or/arginine supplementation, in whom no clinical abnormalities have been observed [16, 26].

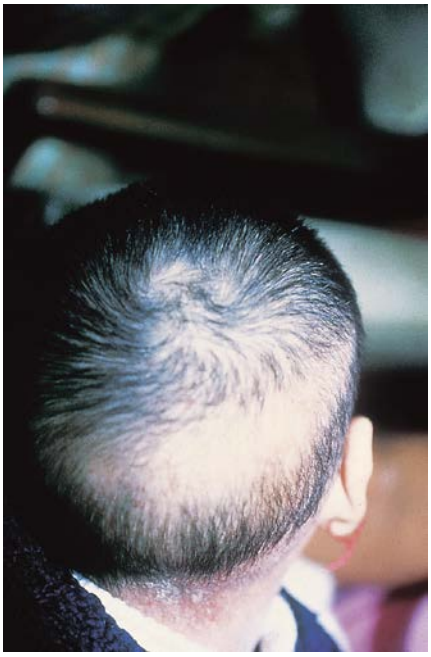

Figure 29.4 CGG: More extensive hair loss at the occipital area of pressure.

Figure 29.5 HH: An infant with argininosuccinic aciduria had brittle hair and alopecia.

The diagnostic metabolic characteristic is argininosuccinic aciduria. Hyperammonemia may be massive in the neonatal form. In patients with variant forms of the disease, it is usually episodic and less dramatically elevated. Plasma concentrations of glutamine and alanine are mostly elevated while plasma citrulline is slightly increased as is urinary orotic acid. Plasma arginine may be low. Argininosuccinic acid is specific and best found

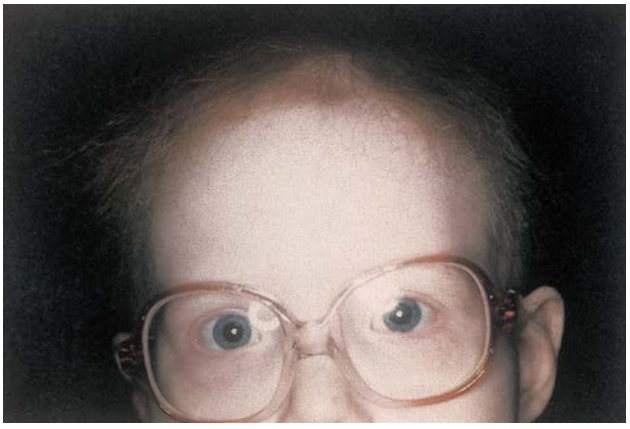

Figure 29.6 RW: A girl with argininosuccinase deficiency [24] who had considerable hair loss during a period of metabolic imbalance. (Illustration kindly provided by Dr EV Bawle of the Children's Hospital of Michigan.)

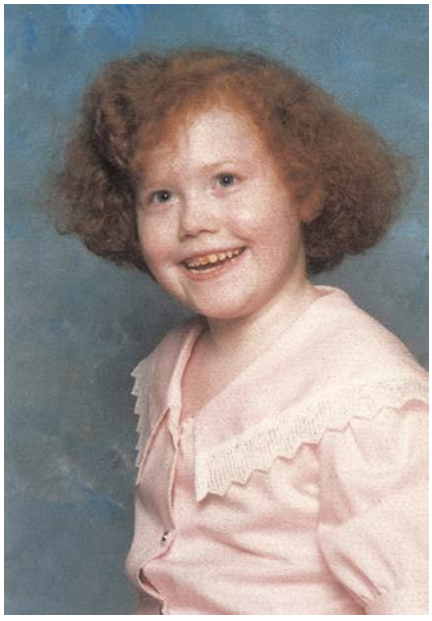

Figure 29.7 RW: When the hair was plentiful. (Illustration kindly provided by Dr EV Bawle of the Children's Hospital of Michigan.)

Figure 29.8 CGG: Trichorexis nodosa.

in the urine. This compound is so efficiently excreted that it may be missed in the blood, as it can also overlie the peak for leucine or isoleucine.. High levels are found in the cerebrospinal fluid. In the urine, argininosuccinic acid is excreted in gram quantities. Values for argininosuccinic acid and its anhydride in the urine ranged from 1163 to 6060 mmol/mol creatinine [19]. However, it may sometimes be missed on routine assays even of the urine for amino acids because the compound is unstable, the peaks occur in a place unfamiliar to the operator, or they may overlap those of other amino acids. The best way to assay for argininosuccinic acid is to boil the urine; this quantitatively converts the compound to its anhydrides, which are then readily seen on the amino acid analyzer [27].

GENETICS AND PATHOGENESIS

Argininosuccinic aciduria is transmitted as an autosomal recessive disease. Its incidence had been estimated to approximate 1 in 70,000 [25]. However, large-scale observational trials in the United States and Europe, as well as implementation of argininosuccinic aciduria in newborn screening programs, have demonstrated that the incidence is as low as 1 in 220,000 newborns [28]. The molecular defect is in argininosuccinate lyase (see Figure 29.1). This enzyme is widely distributed in tissues and can be assayed in erythrocytes, as well as cultured fibroblasts. Argininosuccinate lyase is not only catalyzing arginine production through the urea cycle, but is also part of a multi-protein complex that includes nitric oxide synthase. It is by that essential for nitric oxide synthesis [29]. Enzyme deficiency, therefore, not only results in endogenous arginine deficiency but, in addition, reduces the ability of the cell to use extracellular arginine for nitric oxide synthesis. However, arterial hypertension has not been frequently reported [21, 25], and it remains to be determined whether this complication is still underreported or whether impaired systemic nitric oxide production is restricted to patients with specific mutations.

Deficient activity of the enzyme has been documented in erythrocytes, liver, and fibroblasts [10, 30–33]. In a fraction of its product, it demonstrates alternative splicing, which could be influenced by regional polymorphisms with relevance to enzyme diagnosis [15, 34]. The erythrocyte assay may be misleading; in some patients there may be substantial, even normal, activity even though there is severely defective hepatic activity. The normal enzyme in fibroblasts is immunologically indistinguishable from that of the liver. However, there have been patients reported in whom the activity in fibroblasts was less deficient than that of liver, and others in whom the activity in the liver was less deficient than that of fibroblasts [33, 35]. The activity of the enzyme has often been indirectly assayed by determination of the incorporation of ^{14}C-citrulline into proteins. Assay conditions for the enzyme have been improved by the use of a higher concentration of citrulline. In a group of variant patients, this reduced the relationship to normal from 18–75 percent to 6–28 percent [19]. Higher concentrations stimulated incorporation in normal, but not in mutant, fibroblasts. The method has also been used for prenatal diagnosis. It also appeared to correlate with the variant phenotypes, in which greater activity was demonstrable.

Heterogeneity in the mutations responsible for deficient enzyme activity in argininosuccinic aciduria was demonstrated in complementation studies of fibroblasts of 28 patients [36], in which there was a single major complementation group, but there were 12 interallelic complementation subgroups consistent with 12 allelic mutations. The enzyme is a homotetramer in which the monomeric subunit has a molecular weight of 50 kDa [37]. Immunochemical studies of the enzyme after electrophoresis on sodium dodecylsulfate polyacrylamide gel electrophoresis revealed two bands of approximately 49 and 51 kDa in normal cells [38]. Each of 28 variants had some 49 kDa cross-reacting material (CRM). The 51-kDa band was found in only six variants in which CRM or residual enzyme activity was very high.

These data were consistent with the existence of a number of unique mutations. Some mutations appear at a higher frequency than others and may represent hot spots with higher susceptibility for alteration [39]. Definition of the nature of mutation has supported these conclusions. In four independent cell lines, six mutations were found: three missense mutations, one nonsense mutation, and two deletions [13]. The missense mutations were R111W (arginine 111 to tryptophan), Q286R (glutamine 286 to arginine), and R193Q (arginine 193 to glutamine). In addition, an R95C (arginine 45 cysteine) change was found in a product of consanguinity [14], which when expressed in COS cells exhibited a normal amount of mRNA and only 1 percent of normal enzyme activity. The nonsense mutation changed glycine 454 to X or termination. Two deletions were found that led to skipping of exon 13 [40]. A 13-bp deletion within exon 13 is the most common mutation identified to date, occurring in 8 percent of mutant alleles. The other, a 25-bp deletion, begins at exactly the same spot, which appears to be a hot spot for deletions. The deletions begin with a restriction endonuclease Topo II recognition sequence and start at the Topo II cut site, a site very similar to the ΔF508 deletion in cystic fibrosis and somewhat similar sites in hypoxanthine phosphoribosyl-transferase (HPRT) and β-globin. In a series of five variant patients [19], three novel mutations (R385C in two patients, V178M, and R379C) were detected in homozygous condition. One patient was a compound of R193Q and Q286R. In 27 unrelated patients, 23 mutations were identified [15], 19 of them novel; 15 of 54 alleles contained the IVS5 + 1G > A splice site mutation. In 12 Italian patients, 16 different mutations were found; 14 novel [41]. Genotype phenotype correlations remain elusive [19, 41].

Parents of infants with the disease have been found to have reduced activity of argininosuccinate lyase in erythrocytes and fibroblasts [42]. Prenatal diagnosis may be carried out by analysis of the activity of the enzyme

in cultured amniocytes [42–44]. Contamination with mycoplasma could cause a false-negative result [43]. Prenatal diagnosis in variant families has been accomplished by ^{14}C-citrulline incorporation in amniocytes and chorionic villus cells [19]. The disease may also be detected prenatally by direct assay of the amniotic fluid for argininosuccinic acid [31, 32, 43–45]. It would seem reasonable to always undertake the direct assay in pregnancies at risk. In a family in which the mutation is known, prenatal diagnosis and carrier detection is best carried out by analysis for the mutation. In the Massachusetts program, which depended on paper chromatography of urine at 3–4 weeks of age, eight patients were found in some 600,000 samples indicating the prevalence of one in 70,000 [46]. Of course, some infants may have died earlier. These approaches to newborn screening have been replaced by programs of expanded screening by tandem mass spectrometry [47] with, as yet, unknown sensitivity and specificity of such screening.

The argininosuccinase protein has a structural function first evident from homology with the D-crystallins of avian lens [48, 49]. Duck lens proteins turned out to have enormous argininosuccinate lyase activity. In birds, urea synthesis does not take place; the enzyme is required for the biosynthesis of arginine. In some birds, like chickens, evolutionary divergence has occurred and the major crystallin does not have lyase activity.

TREATMENT

The acute management of the initial hyperammonemic episode and subsequent episodic attacks is set out in Chapter 25, along with principles of the long-term management of hyperammonemic infants. In the acute management of argininosuccinic aciduria, the IV sustaining dose of arginine is increased to 700 mg/kg. After a priming dose of 700 mg/kg, i.e. 8 mL/kg of 10% arginine-HCl, given in 25 mL glucose solution/kg through a central line, the same amount is given as a sustaining dose in maintenance fluid over 24 hours. Because arginine is supplied as the hydrochloride for IV use, blood levels of chloride and bicarbonate are monitored, and hyperchloremic acidosis is treated with sodium bicarbonate. The use of sodium benzoate and phenylacetate may usually be omitted. The infant with argininosuccinic aciduria can be managed, except at times of crisis, using arginine alone and a diet modestly restricted in protein (Figure 29.9). This will be associated with a 2- to 3-fold elevation in plasma arginine and further increases in plasma argininosuccinic acid concentrations.

The principle is that if ornithine molecules can be provided through supplemental arginine in order to keep the urea cycle operating, waste nitrogen can be adequately eliminated in the form of argininosuccinic acid. Argininosuccinic acid contains two more N atoms than the two provided as ornithine, and it is very efficiently excreted, so that it should be as effective as urea in getting rid of

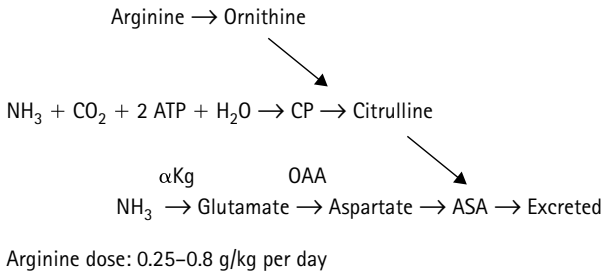

Figure 29.9 Chronic management of argininosuccinic acidemia. Of the four N atoms of argininosuccinic acid, two came from ornithine supplied as arginine. Thus, there is a net loss of two N for every molecule of argininosuccinic acid excreted, which is as efficient as urea. Continued supply of ornithine permits this minicycle to continue to operate. CP, carbamyl phosphate; αKg, α-ketoglutarate; OAA, oxaloacetate; ASA, argininosuccinate.

unwanted nitrogen, as long as there is a supply of ornithine to keep the cycle moving. As in other urea cycle disorders, an objective of therapy is to keep the levels of glutamine in normal range. Increases in ammonium concentrations have been found to lag by days to weeks behind elevations in glutamine [50]. Therefore, periodic measurement of plasma amino acids (including glutamine) and ammonium may permit adjustment of therapy before clinical symptoms appear. Arginine therapy should be sufficient except at times of catabolism, such as during intercurrent infection. Doses of arginine employed have ranged from 0.25 to 0.89 g/kg per day. If the patient is vomiting or cannot take oral arginine, admission to hospital for parenteral arginine is mandatory.

Prognosis for intellectual development probably depends on the nature of the initial hyperammonemia, especially its duration [51] or the nature of recurrent episodes. Children in neonatal hyperammonemic coma for less than three days have a far better outcome than those in a coma for longer periods of time. One should expect patients rescued from neonatal hyperammonemia to have impaired mental development. Over 75 percent have mental retardation, and there is high comorbidity with cerebral palsy, seizure disorders, and visual deficits. The mean IQ reported was approximately 50 [51]. The ability to prevent hyperammonemia by early treatment in patients diagnosed prenatally or during newborn screening should be consistent with a better prognosis. However, some patients may already present in metabolic crisis before the results of newborn screening are available; other patients identified by newborn screening may have a benign disease course [52].

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30

Argininemia

MAJOR PHENOTYPIC EXPRESSION

Mostly neurologic with progressive spastic quadriplegia, psychomotor impairment, convulsions, and microcephaly, varying hyperammonemia, hyperargininemia, argininuria and secondary cystinuria, lysinuria and ornithinuria, orotic aciduria, and deficiency of arginase.

INTRODUCTION

Argininemia is a disorder in which the clinical picture is quite different from the other disorers of the urea cycle. The picture is mostly that of progressive spastic diplegia or quadriplegia [1–4]. It was reported in 1965 by Serrano [1] and in 1969 by Terheggen and colleagues [2]. Evidence of protein aversion, often associated with anorexia, vomiting, and irritability is common in early childhood. The disease is caused by a virtually complete absence [5, 6] of the activity of arginase 1 (EC 3.5.3.1) (Figure 30.1). The human and rat genes have been cloned [7, 8]. The human gene *ARG1* is located on chromosome 6 at band q23.2 [8]. Mutations have been heterogeneous [9–11] with correlation between the severity of the mutations and the degree of clinical symptoms [12, 13].

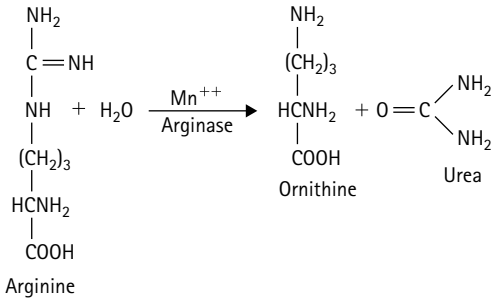

Figure 30.1 The reaction catalyzed by arginase.

CLINICAL ABNORMALITIES

Patients with argininemia are often recognized as abnormal because of failure to pass developmental milestones. With the advent of spasticity or opisthotonus, they may be first thought to have cerebral palsy [3, 4, 12–20]. Onset may be with convulsions in the neonatal period [1–3]. Some patients may have recurrent cyclic vomiting from the early days of life [21]. Others may display anorexia, irritability, or inconsolable crying; some patients have failure to thrive and to grow [4]. Alternatively, there may be no signs in early infancy until it is apparent that development is delayed. The mother of one infant remarked on her drowsiness after feeding [3]. Protein intolerance has been observed very early in life [19].

In the established phenotype, the patient has marked spasticity and is frequently opisthotonic (Figures 30.2 and 30.3). If walking is possible, the gait is a spastic toe-walk. Scissoring of the lower extremities is common. Muscle tone is hypertonic, and the deep tendon reflexes are accentuated, both usually more so in the legs than in the arms. Patients may be hyperactive or irritable. They may be ataxic or appear clumsy. Involuntary movements may be choreic or athetoid, or there may be tremors. Drooling and dysphagia are common. There is often a long period between the manifestation of the first classical clinical symptoms and age at diagnosis. The following features may help to distinguish argininemia from cerebral palsy: patients show (1) progression of spasticity, (2) deterioration of cognitive and language function, (3) avoidance of high-protein foods, and (4) usually absence of a clear history of hypoxia at birth or

Figure 30.2 TG: A 19-year-old girl with argininemia. She walked with a distinct spastic gait and had equinovarus posturing of the feet. Deep tendon reflexes were brisk and there was clonus at both ankles.

during the neonatal period [4]. Differentiating argininemia from hereditary spastic paraplegia is more difficult, but the following clinical features may point to arginase 1 deficiency: (1) progression of spasticity, (2) avoidance of high-protein foods, and (3) infrequent hypertonic urinary bladder disturbances. Convulsions are regularly observed and abnormalities of the electroencephalogram (EEG) are the rule [22]. The pattern of the EEG may be that of a spike and wave. Patients often develop microcephaly (Figure 30.4) and cerebral atrophy is visible on computed tomography (CT) or magnetic resonance imaging (MRI). Psychomotor impairment is usually severe, but it may be minimal in patients diagnosed and treated early.

Patients with argininemia may have episodic vomiting and hyperammonemia. The clinical features may include progressive gait abnormalities due to spasticity, as well as acute episodes of ataxia, behavior disturbances (aggression, hyperactivity, and irritability), vomiting, lethargy, and seizures. These episodes are often precipitated by intercurrent viral illnesses and are usually associated with moderately elevated plasma ammonia concentrations of 3 to 4 times normal and plasma arginine concentrations greater than 5-fold normal, often exceeding 1000 μmol/L (normal less than 120 μmol/L). Symptomatic hyperammonemia can progress to coma, and death in infancy has been reported

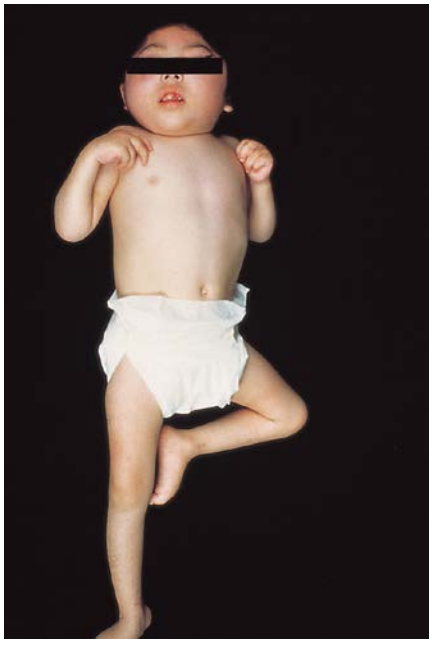

Figure 30.3 A patient with argininemia has spasticity, often opisthotonic, had severely impaired mental development and microcephaly. Convulsions began at 23 days of age. By four years, she had no head control. (This illustration was kindly provided by Dr Makato Yoshino of Kurume University School of Medicine, Kurume, Japan.)

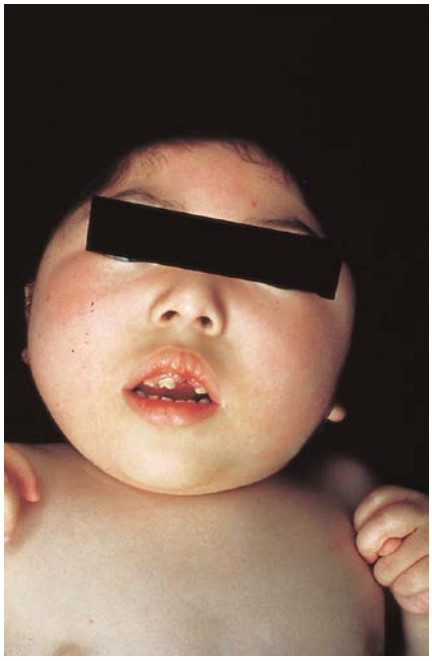

Figure 30.4 The microcephaly of the patient. Neuroimaging revealed cerebral atrophy. Concentrations of arginine ranged from 3.1 to 19.4 mg/dL. Orotic aciduria was 6900 mg/mg creatinine. (This illustration was kindly provided by Dr Makato Yoshino of Kurume University School of Medicine, Kurume, Japan.)

[19, 23]. In one patient, hypertonicity, tachypnea, lip smacking, and right-sided bicycling movements at 30 hours heralded fatal cerebral edema [23]. We have seen severe hyperammonemic coma in a neonate with low concomitant levels of plasma arginine mimicking the biochemical constellation of OTC deficiency (Chapter 26). He was treated successfully as such and the diagnosis unmasked a few months later, when arginine proved to be constantly elevated even without supplementation. In a report [24] of an infant who presented at five months with an ammonia concentration of 736 μmol/L and cerebral edema, experience was reported with eight other patients with recurrent ammonia elevation. Following acute hyperammonemic episodes, neurologic function may further deteriorate.

Plasma ammonia concentrations are usually normal when argininemic patients are well; however, glutamine concentration may be increased. Interestingly, increased plasma arginine concentrations are often relatively constant for most patients, barely responding to protein intake variation within a normal and growth sustaining range. In some patients, the levels of ammonia were elevated in both the fed and fasting states [2]. In an occasional patient, the concentration of ammonia may be surprisingly high in the absence of obvious symptoms of hyperammonemia [3]. On the other hand, in the patient with neonatal cerebral edema, the peak ammonia was only 114 mmol/L, and it fell to 18 mmol/L with only supportive therapy [23]. There may be hepatomegaly.

One patient was first diagnosed at 18 years of age when he developed hyperammonia after initiation of treatment for seizures with valproic acid [25]. Hyperammonia after valproic acid has also been observed in ornithine transcarbamylase deficiency (Chapter 26) and citrullinemia (Chapter 28). We were involved in a peracute death in hyperammonemic coma of a female adolescent with supposedly stable ornithine transcarbamylase deficiency in whom valproate therapy was started. Valproate should never be initiated in any patient with a known urea cycle disorder.

Serum activities of the transaminases may be elevated and the prothrombin time may be prolonged. Unusual zones of different coloration and fragility of the hair have been reported in one patient [1]. Biopsied liver has been reported [5] to reveal multifocal hydropic changes.

Patients are generally recognized by the assessment of the concentration of amino acids in the blood or urine, the latter of which may be mistaken to be that of cystinuria. All such abnormal findings should be pursued by the quantitative analysis of amino acids in plasma. In patients with argininemia, the plasma concentration of arginine is usually four to 20 times that of the normal individuals, often up to 1500 μmol/L [15]. However, in one patient with very severe deficiency and lethal clinical disease the value was only 170 μmol/L [19], and we have even seen concentrations below normal in the initial presentation of hyperammonemic coma in a newborn.

Although death due to argininemia is uncommon, when compared to other urea cycle disorders, morbidity is high. Especially when untreated affected children have progressive intellectual disability, spastic di/quadriplegia, seizures, and impaired language function with worsening or loss of spoken language. Without therapy, patients usually do not reach normal adult height. Secondary complications, such as joint restriction and loss of ambulation, develop in almost all patients [4]. Brain neuroimaging studies generally show microcephaly and cortical atrophy, with enlargement of the lateral ventricles and sulci. Moreover, a variable degree of cerebral and mild cerebellar atrophy has been described in a series of patients [26]. Concentrations of arginine in cerebrospinal fluid (CSF) are also markedly elevated [3]. The concentration of the glutamine is also often increased, especially in the acute hyperammonemic crisis. In the neonate with cerebral edema [23], the plasma glutamine was 909 mmol/L and that of the CSF was 9587 mmol/L. Concentrations of other amino acids may also be elevated in the CSF [5, 20]. These include ornithine, aspartate, threonine, glycine, and methionine. A mechanism for their increase is not clear. The excretion of arginine in the urine is substantial. The urine also contains increased quantities of lysine, cystine, and ornithine; this is the result of competition for their renal tubular reabsorption by the large amounts of arginine being processed by the kidney [22]. The amounts of cystine and ornithine are usually relatively less than observed in cystinuria. Lowering of plasma concentrations of arginine, by restriction of intake of protein, effectively normalizes this pattern of urinary amino acid excretion.

GENETICS AND PATHOGENESIS

Argininemia is one of the rarest inborn errors of urea synthesis, inherited as an autosomal recessive disease [4, 27]. Data provided by the UCDC calculated the overall incidence of argininemia in the United States as 1 in 950,000 people [27].

The molecular defect is in the enzyme arginase 1. It catalyzes the conversion of arginine to urea and ornithine (see Figure 30.1). Arginase 1, encoded by ARG1 and referred to as the liver isoform, contributes 98 percent of the arginase activity in liver but is also present in red cells. The mitochondrial isoform arginase 2, which predominates in the kidney [28], is encoded by a separate gene loci ARG2 at 14q24.1. Arginase 2 activity becomes elevated in patients with argininemia [29], and it appears possible that the presence of arginase 2 in argininemia provides some degree of protection from nitrogen accumulation, resulting in less severe hyperammonemic episodes than in other urea cycle disorders. The activity of the renal arginase also provides the mechanism for the relatively normal production of urea in these patients. The renal enzyme is 58 percent identical to the hepatic enzyme and 70 percent identical to Xenopus arginase.

The activity of arginase 1 is readily measured in erythrocytes and it is through assay in this tissue that the diagnosis is usually made [3, 5, 14]. The defect has also been demonstrated in liver [6]. The enzyme is not expressed in

cultured fibroblasts [30]. The enzyme from rat liver has been crystallized. It is a trimer of three 35-kDa monomers [31]. Negligible amounts of mRNA are normally found in tissues other than liver and erythrocytes. Western blot analyses of 15 patients were reported to reveal detectable arginase protein in only two patients [9].

The human gene at chromosome 6q23.2 is 11.5 kb in size and contains 18 exons [32]. The crystal structure of the enzyme has been elucidated [31, 33]. A dimagnesium cluster is essential for enzymatic activity and stability of the protein [34]. Mutation analysis revealed no gross deletions by Southern blot analysis in 15 patients [9]. In three, a TaqI restriction enzyme cleavage site was missing. In two of these, mutations were identified. One was homozygous for R291Y (an arginine-to-tyrosine change) and the other heterozygous for T290S (a threonine-to-serine change). A Japanese patient was found to be a compound in which, on one allele, there was a four-base deletion in exon 3, which caused a frameshift at position 87 and a premature termination 45 residues later while, on the other allele, a single base deletion in exon 2 led to a frameshift at 26 and premature termination on five residues later [10]. In another study of Japanese patients [35], the mutations found were in W122X, 6235R, and L282 frameshift. The enzyme activities assayed in expression studies in *E. coli* were zero, consistent with enzyme assays in the erythrocytes of patients. Mutations in *ARG1* though heterogeneous have largely been point mutations and microdeletions rather than major or structural alternations in the gene, indicating extensive genetic heterogeneity [8, 11].

Detection of heterozygotes has been accomplished by finding arginase levels in erythrocytes or leukocytes that were appreciably less than the control levels [18, 24]. The leukocyte concentration of arginine has been used to distinguish heterozygotes when arginase levels were not useful. Mutation analysis is preferred for this purpose when the mutation is known. Prenatal diagnosis has been difficult because the enzyme is not expressed in fibroblasts [30]. Direct measurement by gas chromatography-mass spectrometry (GCMS) of orotic acid in amniotic fluid [36] was not useful in ornithine transcarbamylase deficiency, but it could be more reliable in argininemia. Fetal blood sampling can be employed, but with risk of fetal loss. If the mutation is known, this is the method of choice in prenatal diagnosis.

A screening method was developed that permits routine screening of newborns for deficiency of arginase [37]. The advent of tandem mass spectrometry and its applications to newborn screening has supplanted this approach. However, this methodology is currently not sensitive enough to reliably detect all cases.

In the presence of defective activity of arginase, there is in addition to the accumulation of arginine, an impressive orotic aciduria [5, 38]. The amounts of orotic acid found in the urine are considerably greater than those of patients with argininosuccinic aciduria and occur in the absence of hyperammonemia. So, this is not a consequence of accumulation of carbamylphosphate behind the block, as occurs in ornithine transcarbamylase deficiency. Rather, it appears to be the direct result of the stimulation by accumulated arginine of N-acetylglutamate synthetase (NAGS), which leads to increased synthesis of carbamylphosphate (Figure 30.5) [38]. Arginine is a normal activator of NAGS. This accumulation of carbamylphosphate when arginase is deficient leads preferentially to the biosynthesis of pyrimidines. Consistent with this were the low levels of ornithine reported by Yoshino *et al.* [3], and the fact that, as ornithine levels were increased by treatment, the excretion of orotic acid decreased to normal levels, even though the concentration of arginine rose considerably. The orotic aciduria in this condition is also associated with increased excretion of uridine and uracil [39]. N-Acetylarginine, 2-oxo-guanidinovaleric acid, and argininic acid, direct derivatives of arginine, are also found in the urine in this disorder, as well as guanidinoacetic acid and guanidinobutyric acid, compounds in which the amino group is donated via transamidination reaction [18, 40, 41]. Guanidinosuccinic acid excretion is not increased, whereas it does increase in individuals given an arginine load, suggesting a role for arginase in the generation of this compound [42, 43]. The serum concentration of urea is usually normal in these patients.

The pathogenesis of the neurologic disability in argininemia is not clear, but doubtless it is the result of the chemical milieu in which the patient's brain develops. Intermittent or chronic elevation of ammonia could be sufficient, but the phenotype is so different from that of the other defects of the urea cycle that something about arginine or its products, e.g. the guanidino metabolites [44, 45], must have effects on the central nervous system.

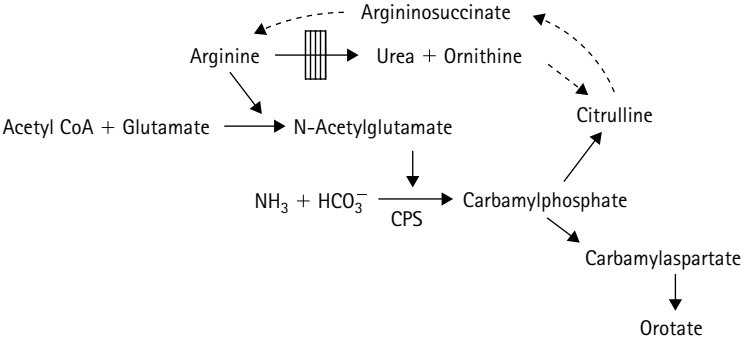

Figure 30.5 Pathogenesis of the orotic aciduria of argininemia. Accumulation of arginine provides an effector function on N-acetylglutamate synthetase, and the product of this reaction stimulates carbamylphosphate synthetase (CPS). The carbamylphosphate generated does not accumulate, and since in the absence of arginase, ornithine is limiting, it flows along the pathway of pyrimidine synthesis to orotic acid.

Neurotransmitter metabolism has been reported to be impaired in argininemia [46]. The production of nitric oxide from arginine could be another factor [29].

The occurrence of high levels of glutamine, especially in the CSF in the neonate with cerebral edema and only modest hyperammonemia [23], is consistent with a role for glutamine in this complication.

An arginase-deficient mouse displayed growth deficiency and hyperammonemia that led to death by 12 days of life. It may therefore not be a suitable model for the study of the long-term effects of arginase 1 deficiency on the brain [29, 47].

TREATMENT

As in other urea cycle disorders, nutritional therapy has been designed to keep levels of arginine within normal limits [48], and success has been reported not only in meeting this objective, but also in promoting normal growth and neurologic development [4, 5, 17, 49–53]. The methods employed have included strict protein restriction [48] and the use of mixtures of amino acids excluding arginine [4, 50]. The latter approach has been effective in controlling levels of arginine in a patient treated from birth [51], as well as in older individuals [4]. Unfortunately, only few patients can adhere to a diet rigorous enough to get arginine concentrations into or near the normal range [52]. Supplementation with lysine raised low levels of lysine in serum, but concentrations of arginine in plasma and CSF increased and concentrations of ornithine in the CSF fell [50]. Supplementation with ornithine improved levels of ornithine and had a pronounced effect in lowering the amounts of orotic acid in the urine [3, 50]. During combined supplementation with lysine and ornithine, a patient gained weight well and epileptiform activity on the EEG improved [50]. In one report [49], nitrogen-free analogs of some essential amino acids were employed to minimize further the nitrogenous sources of arginine in a low arginine diet.

Sodium benzoate therapy was employed in a 15-year-old patient with progressive spastic diplegia and borderline intelligence who had numerous hyperammonemic episodes, and required nasogastric tube feeding to maintain nutrition [53]. The doses employed were 250–375 mg/kg. This approach controlled levels of ammonia and reduced plasma concentrations of arginine. Restriction of the dietary intake of arginine reduced levels further. The excretion of orotic acid decreased to normal levels. Urinary hippurate excreted amounted to 60–80 percent of the administered benzoate and this constituted 35–43 percent of the urinary nitrogen. Progression of the diplegia was thought to have stopped. Sodium benzoate was also efficacious in a 12-year-old patient with less severe disease who had self-selected a diet low in protein [54]. Phenylbutyrate or phenylacetate should have similar effects, but the resulting odor is less acceptable socially.

Phenylbutyrate increases expression of some genes, and it has been reported [55] to increase the activity of arginase in mice and in cultured cells. It was not tested in cells of patients with argininemia, but might be useful in patients with residual activity. Another new therapeutic approach is to reduce guanidinoacetate similarly to the treatment in guanidinoacetate methyltransferase deficiency (Chapter 101). A 9-year-old boy with argininemia and elevated concentration of guanidinoacetate received creatine, L-ornithine, and sodium benzoate along with an arginine-restricted diet. This resulted in a reduction of guanidinoacetate and clinical improvement with reduced seizure frequency and improved alertness [45].

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31

Hyperornithinemia, hyperammonemia, homocitrullinuria syndrome

MAJOR PHENOTYPIC EXPRESSION

Episodic hyperammonemia, ataxia, vomiting, lethargy, or coma; failure to thrive; impaired mental development; seizures; hyperornithinemia; homocitrullinuria; and defective transport of ornithine into mitochondria.

INTRODUCTION

The disorder was first described by Shih *et al.* [1] in a patient with impaired mental development, irritability, and myoclonic spasms, who had intermittent attacks of hyperammonemia and ataxia. He was described by the parents as having attacks in infancy of sudden jumping, as though he had been stuck by a pin. Dropping of the head was a concomitant of these myoclonic spells.

The term "hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome" was coined to characterize the specific biochemical pattern that has been observed. Just over 100 patients [2–5] have been reported since, six from a single, consanguineous kindred [3]. The fundamental defect is an inability to transport ornithine into mitochondria (Figure 31.1) [6–8]. The gene was cloned by Camacho and colleagues [9] and localized to chromosome 13q14.11. Three mutations were found

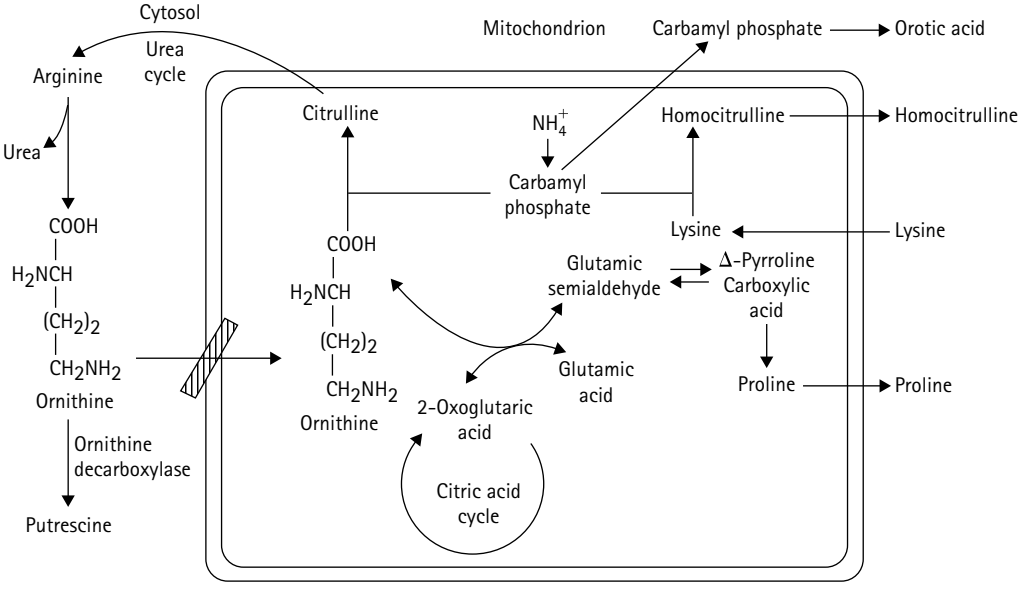

Figure 31.1 Metabolic interrelations involving ornithine. The defect in the HHH syndrome is in the transport catalyzing the movement of ornithine into mitochondria.

to account for 21 of 22 possible mutant alleles including F188D which is common in French-Canadian patients. A second common mutation is p.R179X, prevalent in patients from Japan and the Middle East [10]. An additional mutation T32R was found in two families of Mexican descent with a mild phenotype [11].

CLINICAL ABNORMALITIES

The onset of symptoms in HHH syndrome ranges from the neonatal period (≤28 days) to late adolescence/adulthood (>12 years). Intermittent episodes of hyperammonemia are characteristic features of this disorder. This may be manifest in episodic vomiting, lethargy, coma, or ataxia. It was evident in the experience with the initial and subsequent patients that these symptoms vary directly with the dietary intake of protein and the degree of hyperammonemia. This disease may first present as a Reye-like syndrome. Our patient was diagnosed as an example of Reye syndrome prior to referral, despite the fact that coma developed following feeding of 8 g protein/kg following a thermal burn (Figures 31.2 and 31.3).

Acute neonatal presentation with hyperammonemic coma is less common (22%) than late onset (>28 days; 78%) [5]. In infancy, failure to thrive and developmental delay have been observed, although the initial patient grew along the 10th percentile [1], except when fed his lowest intake of protein, and others have grown normally while receiving diets moderately restricted in protein [5, 6]. In our patient, failure to thrive was associated with very low levels

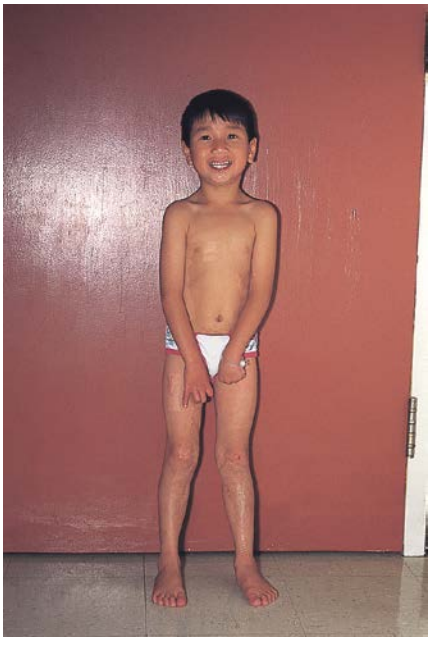

Figure 31.2 A five-year-old Vietnamese boy with HHH syndrome. Scars on his legs signify the thermal burns and its attendant treatment with large amounts of protein that led to his only episode of coma and an initial diagnosis of Reye syndrome.

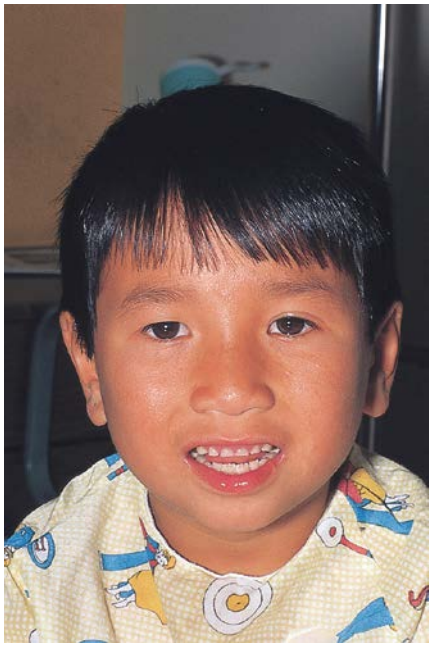

Figure 31.3 Close up reveals no unusual physical features. He was developmentally delayed.

of lysine in plasma. Prior to supplementation with lysine orotate, growth in length had virtually ceased. Growth was rewarding following supplementation, which returned concentrations of lysine in plasma to normal.

Ultimate intelligence has ranged from low normal to severely mentally impaired [1–5, 11–13]. In two families, IQ levels ranged from 76 to 80. In one, diagnosis was made as part of an evaluation for poor school performance in otherwise asymptomatic brothers. In the other family, the diagnosis was made at three years of age on the basis of mild hyperammonemia following gastroenteritis, while the 13-year-old sister was asymptomatic; IQ was 79 in both siblings who were doing well in mainstream classes.

Hyperammonemic attacks may be less frequent in older patients, who may select a diet low in protein. On the other hand, a 21-year-old, with severely impaired mental development continued to have stuporous episodes, at least once a month, which lasted up to two hours. Seizures have been observed with onset from ten months to 18 years. They may be generalized, tonic-clonic, as well as myoclonic [5, 6]. One patient presented with attacks of headache progressive to unconsciousness beginning at 39 years of age [7]. Our patient has been left with a chronic seizure disorder, despite an absence of symptomatic hyperammonemia since the initial episode of coma. Cerebella ataxia has also been observed [5, 10].

As in ornithine transcarbamylase deficiency (Chapter 26), HHH syndrome may present with a hepatitis-like disease or even fulminant liver failure with severe coagulation abnormalities (e.g. subdural hematoma, gingival bleeding, melena) requiring urgent liver transplantation [14]. Elevations of transaminases with or without signs of acute liver failure (i.e. coagulopathy) may occur in the absence of hyperammonemia [5, 15, 16].

Liver changes include vacuolated hepatocytes with intracytoplasmic glycogen deposition, small nuclei, dense chromatin, and fat droplets without fibrosis. Furthermore, mitochondria appear abnormally shaped and sized and contained crystalloid structures [3, 16, 13, 17]. Our patient [7] had hepatic microvesicular fat, which had appeared to confirm the diagnosis of Reye syndrome.

Another especially instructive case is a 3-year-old Italian boy who had developed inconspicuously except for an, in retrospect, moderate aversion to protein-rich food and mild speech delay, when he presented with lethargy during a gastrointestinal infection [18]. During the following four days, he suffered a rapidly progressive hepatocellular necrosis with highly elevated transaminases, coagulopathy (PT ranged from 48% (day 2) to <5% (day 5), normal range 70–120%, and moderate hyperammonemia of at peak 170 μmol/L. Metabolic testing revealed elevated concentrations of ornithine in plasma, homocitrullinuria and orotic aciduria. A defect of the urea cycle was suspected and intravenous supplementation with arginine and protein-restriction started. After approximately 24 hours, blood ammonia had normalized. INR and transaminases both improved over the next days, and the need for liver transplantation was postponed.

Ocular findings, in contrast to gyrate atrophy of the retina, have been normal, except for a patient who developed papilledema during an attack of acute symptomatic hyperammonemia [3]. Another patient had retinal depigmentation and choroidal thinning [19], but visual function was normal.

Progressive spastic paraplegia was emphasized as a clinical characteristic in three patients in one family [12]. It develops in almost all patients and was clearly evident in the oldest patient, who began to have progressive disturbance of gait at 14 years, and at 21 had increased deep tendon reflexes, sustained ankle clonus, and bilateral Babinski responses. His IQ was 67. He stuttered and had an aggressive personality that led to psychiatric consultation. His 18-year-old sister had an IQ of 60 and could not run or jump; deep tendon reflexes were increased and there were ankle clonus and Babinski responses. The 13-year-old brother had brisk deep tendon reflexes and an IQ of 51. Others have been reported with spastic paraplegia [17]. Pyramidal tract signs may be prominent [19].

Cortical and cerebellar atrophy, multiple stroke-like lesions as well as or basal ganglia calcifications have been described on computed tomography (CT) and magnetic resonance imaging (MRI) [7, 17, 19, 20] and there have been abnormal white matter changes. Metabolic abnormality is usually first evident in hyperammonemia. The levels encountered in an acute attack, even in a patient in coma, are usually considerably less elevated than those we are accustomed to in neonatal infants with disorders of the urea cycle. The concentration of ammonia may be chronically elevated in a patient ingesting a diet high in protein. More often, the level is normal in fasting, but elevated postprandially. The concentrations of glutamine and alanine in plasma may be increased as concomitants of hyperammonemia. Orotic acid excretion has been reported to be elevated in only about half of the patients [3], but it may be induced by loading with protein or alanine. Our patient (Figures 31.2 and 31.3) had little, and often no, measurable urinary orotic acid at baseline, but loading with alanine led to a pronounced orotic aciduria (Table 31.1). He was a refugee from Vietnam accustomed to a low protein diet. As he gradually became Americanized, his protein intake increased and the amounts of orotic acid in the urine increased progressively (Table 31.2). The initial diagnosis may be made difficult by the increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) that may occur acutely with the hyperammonemia [1, 11].

Table 31.1 Excretion of orotic acid following alanine[a]

Hours	Mg/g creatine
0.0–2.5	18
2.5–5	236
0.5–6	229
0.6–24	242

[a] The dose of alanine was 400 mg/kg.

Table 31.2 Relationship between the excretion of orotic acid and the intake of protein

Protein intake (g/kg/day)	Urinary orotic acid (mg/g creatine)
2	–
3.7	12
4.7	19
5.4	272

A woman with this disorder treated successfully during pregnancy with arginine to control ammonia delivered a normal baby whose IQ at five years was 130 [21]. Another woman going through three pregnancies experienced nausea, dizziness, unsteadiness with mild hyperammonemia. In the course of her pregnancy, she developed petit mal seizures and at term she delivered a baby who had intrauterine growth retardation. The two other babies were not growth retarded. Within 24 hours of delivery, the woman developed elevated ammonia levels, however, she did not develop hyperammonemic coma and responded well to treatment [22].

GENETICS AND PATHOGENESIS

Hyperornithinemia is a hallmark feature of the metabolic abnormality of this disease which has been found in over 100 patients. Concentrations in plasma have usually ranged from 270 to 780 μmol/L [1–4]. Concentrations as high as 915 and 1439 μmol/L have been recorded [1, 12].

Ornithinuria has ranged from 73 to 8160 μmol/g creatinine. The highest levels of ornithine in body fluids have been those encountered during acute episodes of hyperammonemia. Confronted with an elevated concentration of ornithine and hyperammonemia, especially in a patient with orotic aciduria, one thinks about ornithine transcarbamylase deficiency, but ornithine concentrations are never elevated in ornithine transcarbamylase deficiency, even in those with unusual kinetic properties (Chapter 26). Oral loading with ornithine in HHH syndrome leads to higher peak levels than in normal individuals and a slower return to baseline [1, 12].

There are two other types of hyperornithinemia: one with gyrate atrophy of the choroid and retina, in which the activity of ornithine-5-aminotransferase is deficient [23], and a disorder reported [24] in two siblings with impaired mental development and renal tubular dysfunction, which may represent a partial deficiency of the same enzyme, because its activity in liver was reported to be 60–80 percent reduced, but kinetically normal [25]. In any case, neither of these hyperornithinemic situations is ever hyperammonemic.

Homocitrullinuria is the third major feature of the disease. In the presence of accumulated carbamylphosphate, lysine is carboxylated to form homocitrulline (see Figure 31.1), which is efficiently excreted in the urine. Reported levels of excretion have ranged from 93 to 2380 μmol/g creatinine. As in the case of the orotic aciduria, homocitrullinuria may be absent or not prominent in patients receiving little protein in their diets. Its levels of excretion can be correlated with protein intake [26] or the administration of lysine, and good correlation was observed between the urinary homocitrulline: creatinine ratio and the plasma lysine: ornithine ratio [26]. Homocitrulline is commonly found in the urine of infants and children, a consequence of its formation by the heat treatment of milk products, and its subsequent ingestion and excretion [27, 28]. It is often found in patients with generalized aminoaciduria and regularly follows lysine loading in normal children and adults [29].

Concentrations of lysine in the blood may be elevated during the acute attack of hyperammonemia as a nonspecific concomitant of hyperammonemia. During steady-state conditions, levels of lysine in blood and urine are usually low [12]. We have observed that lysine may become limiting for growth in this disease.

Other unusual compounds may be found in the urine of the patient. Among those identified is 3-aminopiperid-2-one, a cyclic D-lactam or methylester of ornithine [30]. Amounts as high as 459 μmol/g creatine have been reported [12]. Accumulation of ornithine in the cytosol also results in increased levels of polyamines [31], providing a possible link to pyramidal tract damage in argininemia (Chapter 30).

The molecular defect is in the transport system responsible for the movement of ornithine into mitochondria (see Figure 31.1) [8, 26]. This makes ornithine limiting for the synthesis of citrulline and impairs the operation of the urea cycle. This transporter was reported by Gamble and Lehninger [32] to be unidirectional and highly stereospecific for L-ornithine. It requires respiratory energy. The driving force for entry of ornithine is a negative internal transmembrane potential produced by the entry of proton-conducting anions. The system was characterized in rat liver mitochondria; it was not operative in the heart. Citrulline passes through the membrane in both directions without requiring respiratory energy.

Evidence for a defect in the ornithine transport system was first obtained [6] by the study of ^{14}C-ornithine incubation in intact fibroblasts. Similar results were obtained in studies of fibroblasts incubated with ^{14}C-ornithine and assessment of its incorporation into protein [33]. The apparent K_m for this process in a patient's fibroblasts was ten times that of controls. Direct measurement of amino acid concentrations in hepatic mitochondria of a patient revealed the concentration of ornithine to be low [7]. Fibroblasts of patients with this disorder were effectively complemented by those of a patient with gyrate atrophy, while the cells of two patients with the HHH syndrome fell into the same complementation group [33]. Inheritance is autosomal recessive.

The ornithine transporter gene *SIC25A15*, which was called *ORNT1*, was identified by the use of sequences from the *ARG 11* and *ARG 13* genes of *Neurospora* and *Saccharomyces* which encode the mitochondrial carrier family proteins that are involved in the transport of ornithine across the mitochondrial inner membrane [9]. The expression of *ORNT1* is high in the liver. Expression of *ORNT1* in transformed fibroblasts of patients with HHH syndrome restored ornithine transport function. The gene contains seven exons over 23 kb [8]. It encodes a 301 amino acid protein. A 4.2-kb mRNA transcript is expressed in liver and pancreas. The gene was mapped to chromosome 13q14.11 [8, 9].

Among mutations observed [9, 11], F188, a 3-bp inframe deletion in a sequence of four consecutive TTC phenylalanine codons encodes an unstable functionless protein. This mutation was found in nine of ten French-Canadian homozygotes and one heterozygote. E180K encodes a stable properly targeted protein and results from a G to A transition at bp 538. This mutation was found in the patient's Irish-American father, but not in his Japanese mother, who had a terminal microdeletion 13q14. Our patient had a nonsense mutation R179X (Camacho *et al.*, personal communication, 2002). This nonsense mutation p.R179X is commonly found in patients from Japan and the Middle East [10]. In 16 patients from 13 unrelated families with HHH syndrome, 13 different mutations were identified in the *SLC25A15* gene, including 11 novel mutations [16]. In a recent summary, 35 different mutations were listed for 77 patients [5].

A second gene, *ORNT2*, has been discovered [34] which contains no introns and has a structure 88 percent identical to *ORNT1*. It is located on chromosome 5q31.3. Overexpression of protein product *ORNT2* in fibroblasts of patients with HHH syndrome restored the metabolism of ornithine, much as did *ORNT1*. The existence of this redundancy in ornithine transport was considered consistent with the generally milder phenotype in this

disease than in other urea cycle abnormalities, as well as the level of residual ornithine transport observed in cultured F188D and E180K cells. This conceptualization has been strengthened and amplified by the finding that the Mexican families with mild phenotype had a T32R mutation in *ORNT1*, but they were also heterozygous for the glycine 181 polymorphism in *ORNT2*, which is a gain of function variant [11].

A polymerase chain reaction (PCR)-based method for the detection of the F188d mutation has been employed for newborn screening in Northern Saskatchewan, where HHH syndrome is found in high incidences [35]. This population is 94 percent Aboriginal, and it is isolated. Heterozygote frequency was one in 19 live births, resulting in a frequency of 1:1500 affected homocygotes. Ornithine levels in the newborn period were normal in all three populations: mutant, carrier, and normal; thus, analysis for the gene is the only one that is useful for newborn screening.

TREATMENT

Dietary treatment is the essential anchor point of long-term management and requires the knowledge of a specialist metabolic dietitian. A mix of intact dietary protein and medical foods composed of essential amino acids may be necessary. Restriction of the dietary intake of protein to 1.5 g/kg permitted maintenance of the blood ammonia at 90–100 mg/dL and the avoidance of acute attacks of hyperammonemia, whereas 2 g/kg led to symptomatic hyperammonemia [1]. In this patient, an acute load of 100 mg lysine/kg did not increase homocitrulline excretion. Supplementation of the diet with 1 g lysine hydrochloride increased its blood concentration to a low normal level, but did not appreciably change the plasma ornithine or ammonia in this 19-month-old patient. Supplementation with 1 g ornithine hydrochloride per day increased the plasma concentration of ornithine, but had no effect on the postprandial ammonia or the plasma lysine. On the other hand, supplementation with 6 g/day of ornithine hydrochloride or 7.5 g of arginine hydrochloride in a 32–45 kg woman were reported to lower postprandial ammonia and urinary homocitrulline [13]. In this patient, supplementation with 6 g/day of lysine hydrochloride increased the excretion of homocitrulline. Supplementations of L-arginine or L-citrulline aim at maximizing ammonia excretion through the urea cycle. In patients with creatine deficiency, additional creatine supplementation is recommended [36].

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32

Lysinuric protein intolerance

MAJOR PHENOTYPIC EXPRESSION

Failure to thrive; episodic hyperammonemia; vomiting; diarrhea; failure to thrive; decreased growth; osteoporosis; hepatosplenomegaly and muscular hypotonia in childhood; later on pulmonary interstitial fibrosis and respiratory insufficiency; nephritis; low concentrations of lysine and other dibasic amino acids in plasma; massive excretion of lysine in the urine along with increased excretion of ornithine and arginine; orotic aciduria; and decreased cellular transport of cationic amino acids, resulting from mutations in the gene *SLC7A7* for the amino acid transporter.

INTRODUCTION

Lysinuric protein intolerance (LPI) was first described by Perheentupa and Visakorpi [1] from Finland in 1965, in a report of three patients with familial intolerance to protein and abnormal transport of the basic amino acids. The disease is prevalent in Finland, where it has been estimated to occur in one in 60,000 [2], and Finns or Finnish Lapps have comprised nearly half of the patients reported [1–5]. Later, a similar cluster and incidence was identified in northern Japan [6]. However, the disease may be found in any ethnic population. The fundamental defect is an abnormality in the transport of basic amino acids in the basolateral or antiluminal membrane of epithelial cells (Figure 32.1) [7–9]. The abnormality is in the efflux of these amino acids and can be demonstrated in cultured fibroblasts [10, 11]. The gene for the transporter has been mapped to chromosome 14q11.2 [12]. Founder mutations, c.895-2A>T, a splice-site acceptor change leading to a frameshift and a premature termination, in the Finnish population [13] and R410X in Japan [6], were found in the *SLC7A7* gene. Mutations in other populations are diverse, and more than 50 mutations have been found worldwide [14, 15].

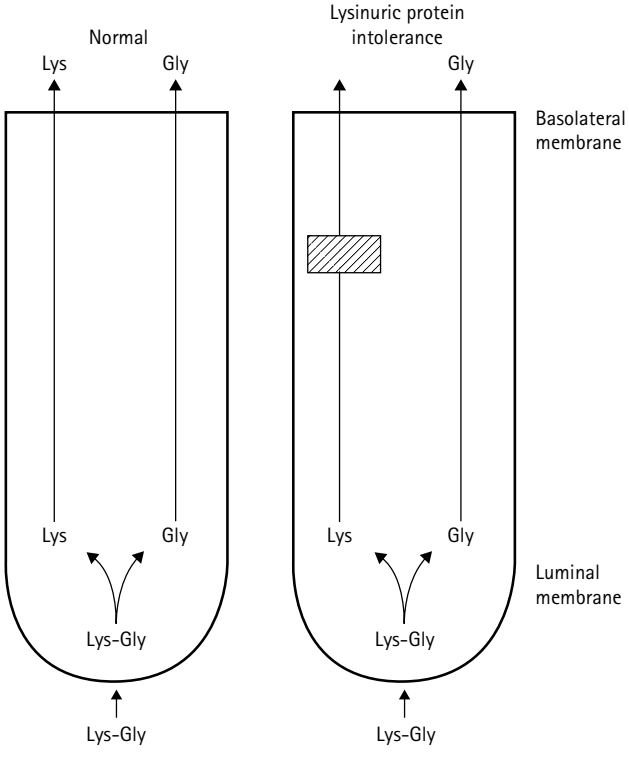

Figure 32.1 Demonstration of the site of the defect in LPI at the antiluminal border of the epithelial cell of the jejeunal mucosa by the administration of a lysylpeptide [5].

CLINICAL ABNORMALITIES

Most infants present with anorexia, vomiting and finally full-blown failure to thrive (Figures 32.2, 32.3, and 32.4) [3]. There may be alopecia. Subcutaneous fat is

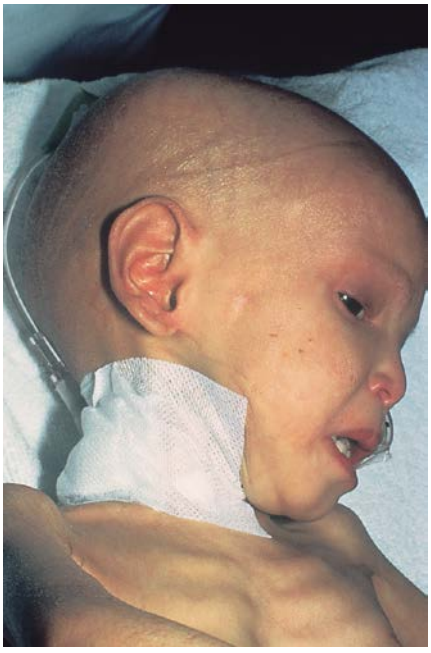

Figure 32.2 RQ: A patient with LPI who presented at five years of age with failure to thrive, alopecia, and skin lesions reminiscent of kwashiorkor or zinc deficiency. Treatment with citrulline reversed the findings in the skin and hair.

diminished or absent, and the skin folds loose. Diarrhea may suggest a malabsorption syndrome. Skin lesions may resemble those of kwashiorkor or acrodermatitis, and zinc deficiency (Figures 32.2 and 32.3). A dry scaly rash is sometimes seen, as well as sores on the sides of the mouth [16]. Dystrophic nails may contribute to the picture of acrodermatitis enteropathica (Figure 32.4). A five-year-old boy with chronic diarrhea and pitting edema of the lower extremities was thought to have celiac disease because villous atrophy was found on intestinal biopsy [16].

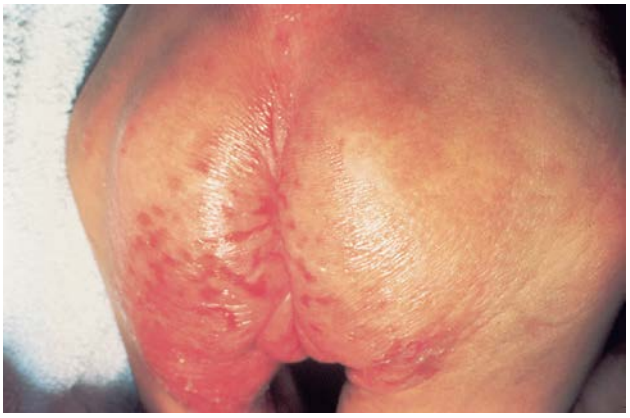

Figure 32.3 RQ: The perianal dermatitis was classic for a diagnosis of acrodermatitis enteropathica. Treatment of the failure to thrive with parenteral alimentation led to hyperammonemic coma. Levels of alanine and glutamine were elevated.

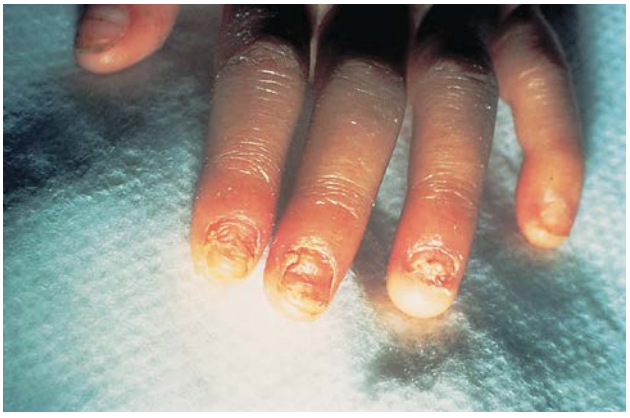

Figure 32.4 RQ: The fingers and nails were also reminiscent of acrodermatitis enteropathica. The day after initiating citrulline, this bedridden patient was sitting up. She ultimately developed nails and hair.

There was no improvement with a gluten-free diet. There is usually some hepatomegaly and muscular hypotonia. The spleen may be palpable. Body weight is reduced, and linear growth falls off. Of 20 Finnish patients [6], 16 had heights that were 2–6 SDs below the mean. Head circumference is normal. Skeletal maturation is usually delayed. Anemia is the rule, leukopenia common, and serum ferritin highly elevated. Increased plasma concentrations of cholesterol and triglycerides are frequently increased [17]. As in protein-deficient malnutrition (Kwashiorkor), there is fatty degeneration, and inflammation of the liver.

As lysine is essential for collagen formation, a deficiency of lysine as in LPI impairs collagen and bone formation leading to osteoporosis and pathologic fractures often already before five years of age [18–20]. Two-thirds have had fractures, usually of long bones or vertebral compression, and often after minimal trauma [19, 20]. Compression fractures of the vertebrae may lead to deformity. Metabolism of calcium and phosphate is normal, but hydroxyproline excretion in the urine is elevated.

Hyperammonemia is usually manifest as episodic attacks of vomiting. These may begin in neonatal infancy or be delayed even until adult life in patients with well-developed aversion to protein [5, 21]. Refusal to eat meat or dairy products has also been observed in patients who have not experienced hyperammonemia [14]. The avoidance of protein-containing foods is an early characteristic, which may begin as early as 12 months of age. Vomiting may be associated with dizziness or headaches. There may be loss of consciousness [22] or even deep coma with an isoelectric electroencephalograph (EEG), especially in patients administered large amounts of protein by gastric tube [5, 21, 23]. Episodic psychiatric symptoms have been observed. Some patients have been mentally impaired, some severely so [24], but most are not. Nonprogressive, asymptomatic opacities have been observed in the lens of the eye [25].

A group of patients, from southern Italy, in which consanguinity was high, has been described [26] with

unusual complications. Manifestations in the patients included abnormalities of the bone marrow, in five of the six examined, in which large cells resembling sea-blue histiocytes suggested a diagnosis of Niemann-Pick disease, but sphingomyelinase was negative, and there was also prominent erythrophagocytosis. Erythrophagocytosis and immunologic abnormalities were also described in another patient with this disease [27]. As there was also high increases of serum levels of ferritin, lactate dehydrogenase, cytokines as well as soluble interleukin-2 receptor, Duval *et al.* [28] have concluded that this is a regular feature of the disease and that LPI should be investigated in patients suspected for familial hemophagocytic lymphohistiocytosis (HPLH1). This hematologic picture has been seen along with the pancytopenia of propionic acidemia [29]. It has also been observed in carnitine palmitoyl transferase I deficiency [30] and in hemochromatosis [31]. Two patients had clinical pancreatitis [26]; in one who required surgery, pancreatic fibrosis was found, indicating chronic pancreatitis, as well as acute liponecrosis.

Very worrisome are the number of late complications that have been described. Some of these patients have presented first in adult life with interstitial disease of the lung or with renal disease.

Pulmonary disease has emerged as a major complication [26, 32, 33]. Pulmonary features are prominent, and they often represent the cause of death. Of 14 patients whose records were reviewed, ten had respiratory abnormalities. In fatal disease, alveolar proteinosis was common. Heterogeneity of pulmonary presentations has been the rule even within a single family. Pulmonary fibrosis is another common presentation. The severity of fibrosis did not correlate with part of alveolar proteinosis. The phagocytic activity of alveolar macrophages was severally impaired.

Imaging of the lungs revealed thickened intrabobular septa and tissues, as well as cysts and ground glass opacity. High-resolution computed tomography has been recommended for the detection of these abnormalities [33].

Respiratory insufficiency or clubbing may be the presenting complaint [33]. Cough, dyspnea, and hemoptysis may occur, and there may be intermittent fever or pulmonary infection. Roentgenograms show reticulonodular interstitial densities. Biopsy of the lung may show cholesterol crystals or granulomas [33] or alveolar proteinosis. Elevated concentrations of cationic amino acids in bronchoalveolar lavage fluid suggest that transport may also be abnormal in pulmonary epithelium [34]. In a number of patients with no pulmonary symptoms, there was roentgenographic evidence of pulmonary fibrosis [32].

In a study on 39 Finnish patients, proteinuria and hematuria were observed in 74 per cent and 38 percent, respectively. Elevated blood pressure, mild to moderate renal insufficiency and, in some cases, end-stage renal disease and chronic renal failure were reported [35], with proteinuria and progressive glomerular and tubular insufficiency [26, 35]. Disease consistent with systemic lupus erythematosus has been reported [36], and the pulmonary disease may be a manifestation of immune complex problems. A full Fanconi syndrome with clinical rickets and deformities may occur [26]. This may obscure the diagnosis because of a massive generalized aminoaciduria. Calculation of the renal clearances of cationic amino acids (lysine, arginine, and ornithine) helps to clarify the urinary loss of these amino acids. Mean values and ranges of the renal clearances of cationic amino acids in patients with LPI are reported [37]. Oral loading with lysine or arginine to test intestinal absorption may be required for diagnosis in such a patient.

Some children and some adults have developed terminal hepatic insufficiency. Pathology was that of extensive fatty degeneration and micronodular cirrhosis [38, 39]. In other patients, biopsy of the liver has been normal [3, 11] or there have been fat droplets in hepatocyte cytoplasm. At autopsy in the adult, changes were noted in the glomerular basement membrane, and immunofluorescence positive for IgA indicated an active glomerular lesion [39]. Terminal micronodular cirrhosis and pulmonary alveolar proteinosis were also found at autopsy.

GENETICS AND PATHOGENESIS

The disorder is inherited in an autosomal recessive pattern [2]. Many examples have been found of multiple affected individuals in a family [21, 22, 24, 40].

The defect in amino acid transport is often first evident in the analysis of amino acids in the blood. Plasma concentrations of lysine, ornithine, and arginine are low. The mean concentration of lysine in 20 patients [37] was 70 mmol/L; those of ornithine and arginine were 21 and 27 mmol/L, respectively. The diagnosis may not be clear from these levels, because the normal ranges can be that low. However, the plasma concentration of citrulline is impressively elevated. The mean was 232 mmol/L [37], which is about four times the upper limit of normal. Concentrations of glutamine and alanine are also elevated, consistent with chronic excess of ammonia. Concentrations of glycine, serine, and proline may also be elevated.

The urinary excretion of lysine is massively increased, and there is increased excretion of arginine and ornithine. The mean excretion of lysine per 1.73 m^2 body surface in 20 patients [37] was 4.13 mmol/24 hours; those of ornithine and arginine were 0.11 and 0.36 mmol, respectively. The renal clearance of lysine was 25.7 mL/min per l.73 m^2; those of ornithine and arginine were 3.3 and 11.5 mL/min per 1.73 m^2. Citrulline and glutamine are excreted in large amounts, but their clearance is normal. Defective renal tubular reabsorption has been demonstrated and is most marked in the case of lysine, less so for arginine, and least for ornithine [41]. The abnormal pattern of urinary amino acids may be elusive, especially when quantification is not employed, at times of very low plasma levels of the basic amino acids and rigid restriction of the dietary intake of protein. Increasing the plasma concentration of lysine clarifies the diagnosis.

Absorption of basic amino acids is defective in the small intestine [5, 42–44], as well as in the renal tubule. In an interesting assessment of mechanism, the oral administration of lysylglycine to patients led to an increase in plasma concentrations of glycine, but not of lysine, while in controls both increased (see Figure 32.1) [5]. Thus, the lysine dipeptide was normally absorbed by patients across the luminal membrane and hydrolyzed intracellularly, but efflux of the lysine, though not of the glycine, was blocked at the antiluminal membrane. *In vitro* studies of biopsied jejunum confirmed this position of the defect [8]. This is very different from cystinuria, where the defect is in the luminal membrane.

The defective transport in LPI is expressed in cultured fibroblasts [9]. Labeled lysine and the nonmetabolizable analog, homoarginine [44], were found not to display the trans-stimulated efflux that occurs in the presence of a cationic amino acid on the other side of the membrane [9, 45, 46]. Heterozygotes were found to display approximately 50 percent of control activity [9].

The mechanisms by which hyperammonemia occur are not completely understood. Concentrations of ammonia are normal in the fasting state, but increase up to 500 μmolar postprandially [3, 11]. Persistent hyperammonemia may result from a large protein intake, prolonged fasting, or infection. The urinary excretion of orotic acid is usually elevated in patients [47, 48] even when they are receiving diets restricted in protein, and there is a major increase after the administration of a protein or alanine load [48]. Levels of orotic acid in urine are helpful in tailoring therapy. The concentration of urea in these patients is usually low.

Abnormal function of the urea cycle is thought to result from intramitochondrial shortage of ornithine, as in HHH syndrome (Chapter 31). In LPI, intravenous (IV) infusion of ornithine or arginine (an ornithine source) prevents the hyperammonemia of protein or alanine loading [1, 3, 11]. Infusion of citrulline also accomplishes this effect and, furthermore, it is effective orally [4, 43]. Oral arginine and ornithine are less effective because they are so poorly absorbed in this condition that supplementation leads to diarrhea [8, 44]. Citrulline is a neutral amino acid and is absorbed via a different transport system, but once in the cell, it is converted via arginine to ornithine. The nature of the defect in efflux would make for high intracellular concentration of ornithine, but the trans-stimulated system has not been found in hepatocytes [49, 50], so hepatic cells would be expected to reflect the depletion of dibasic amino acids evident in the plasma. Some clinical manifestations, such as failure to thrive, anemia, hepatomegaly, and osteoporosis, could be a function of a shortage of lysine. A further pathophysiologic link is to arginine as the starting point of creatine and NO synthesis [51].

The protein affected in LPI is an essential part of system y^+L, transporting cationic amino acids at the basolateral membrane of enterocytes and renal tubular cells. It was shown to be markedly reduced in monocytes and alveolar macrophages from a patient with LPI [52]. This could explain the pathogenesis of the severe complications of LPI including those affecting lung and kidney. Arginine, which is deficient in LPI due to defective intestinal uptake and renal reuptake, may actually be accumulating intracellularly and overloading the NO pathway [51]. As a consequence, lower supplementation doses of citrulline are now recommended, because citrulline is readily converted into arginine.

The molecular locus for LPI was assigned to chromosome 14q11.2 in a study of 20 Finnish families [12]. The gene for the carrier protein SLC7A7 (solute carrier family 7, member 7) maps to this site, and a search for mutations in the Finnish families yielded a mutant allele (1181-2A>T) in which an A to T transversion at 22 of the acceptor splice site in intron 6 leads to altered splicing deleting 10 base pairs and a frameshift [13]. A common haplotype was consistent with a single founder mutation. This mutation was found independently by Italian investigators [53] who also found a frameshift mutation in Italian patients resulting from homozygosity for a 4-bp insertion (1625 ins ATAC). A 543-bp deletion was found in another Italian proband. A nonsense mutation (R410X) is another founder mutation from northern Japan [6]. To date more than 50 pathogenic, mostly private mutations have been identified in LPI, missense and nonsense mutations, insertions, splicing mutations, deletions, and large genomic rearrangements [14, 15, 54–56]. Deletions and large genomic rearrangements amount to 15–20 percent of mutations outside the populations with founder mutations. Expression studies of mutations have revealed proteins that failed to localize to the plasma membrane, as well as proteins that localized but failed to function. Quite different clinical phenotypes have been observed in individuals with the same genotype and genotype–phenotype correlations have been elusive [14], but large deletions involving exons 4–11 and 6–11 had very severe clinical phenotypes [15].

TREATMENT

The effects of citrulline in this condition have formed the basis for effective therapy [4, 11, 43]. Citrulline is provided in doses of 100 mg/kg/day, usually divided into three to five doses, especially with meals. Citrulline supplementation produces an adequate quantity of urea cycle intermediates, and, in this way, prevents hyperammonemia. Protein intake is moderately restricted, a process most patients have begun spontaneously. Intakes of 1–1.5 g/kg per day in children and 0.5–0.7 g/kg in adults have been employed.

In an acute crisis of hyperammonemia, protein intake is stopped, and energy is supplied as IV glucose. Infusion of arginine, ornithine, or citrulline should be effective. Dosage recommended has been 1 mmol/kg as a primary dose in 90–120 minutes followed by 0.5 mmol/kg per hour until symptoms are eliminated. IV sodium benzoate or phenylacetate, or both, may be employed as adjunctive therapy [57].

Lysine depletion may be improved with supplemental L-lysine-HCl (0.05–0.5 mmol/kg, three times per day) [58], but this is limited by malabsorption and intestinal tolerance. ε-N-Acetyllysine has been shown to increase plasma concentrations of lysine [59]. Increase may also be accomplished by the IV administration of lysine [60].

Pulmonary disease may be effectively treated with high-dose regimens of prednisolone [43], but some patients have not responded. Repeated whole-lung lavages are a successful approach for pulmonary alveolar proteinosis [61]. Heart-lung transplantation has not been recommended, because recurrence of disease in transplanted lungs has been reported [62]. Effective treatment of renal complications has not yet been reported.

Elevated levels of cholesterol and triglyceride were documented in 39 Finnish patients [17] whose fat intake was no higher than the general population. Successful lowering was obtained with statin therapy. The authors recommended atorvastatin over others because of more effective reduction of both cholesterol and triglyceride.

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33

Glutamine synthetase deficiency

MAJOR PHENOTYPIC EXPRESSION

Prenatal onset malformations of the brain, seizures, necrolytic erythema of the skin, enteropathy with diarrhea, early neonatal death, and multiorgan failure or chronic encephalopathy and developmental delay, hyperammonemia, low concentration of glutamine in plasma and cerebrospinal fluid (CSF), and deficient activity of glutamine synthetase.

INTRODUCTION

Congenital deficiency of glutamine synthetase was first described by Häberle and colleagues in 2005 [1, 2] in two unrelated newborn infants. Each was the product of consanguineous Turkish parents. They died at two days and four weeks of life respectively. One had micromelia and both had malformations of the brain. So, this disease can be added to those inborn errors of metabolism that express prenatally with congenital malformation syndromes. The disease can also be added to the list of rare disorders that lead to defective synthesis of an amino acid.

Concentrations of glutamine were low in plasma, urine, and CSF. Concentrations of ammonia were moderately elevated. Deficient activity of glutamine synthetase (Figure 33.1) was found in immortalized lymphocytes derived from the initial patient [2]. Each infant was homozygous for an arginine to cysteine substitution in exon 6, p.R324C and p.R341C. A third patient has been reported [3] with a somewhat more attenuated course who had a p.R324S mutation. Glutamine synthase is the only reaction in which glutamine is synthesized. So, its deficiency makes glutamine an essential amino acid.

CLINICAL ABNORMALITIES

The first patient had to be resuscitated at birth, required ventilator support, was flaccid, and showed no signs of development. He died of cardiac arrest at two days of life. The second patient had convulsions and respiratory failure requiring mechanical ventilation. She had voluminous stools and lost weight with enteral feeding; parental nutrition was instituted, but she developed hyponatremia. The third patient was alive at four years of age but continued to have chronic encephalopathy and seizures.

Patient one had polyhydramnios. Congenital malformations included micromelia. Head circumference at 34 cm was in the 75th to 90th percentile, while length and weight were 3rd to 10th percentile. There were flexion contractures at the elbows and knees camptodactyly, ulnar

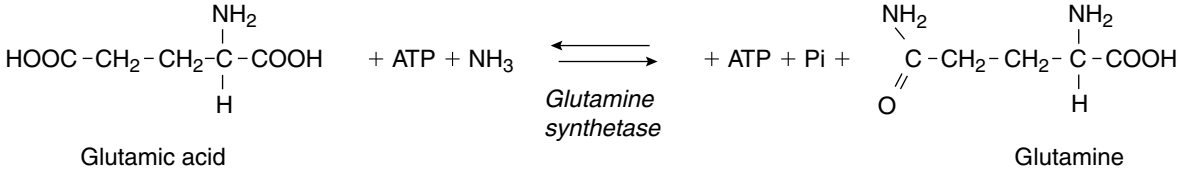

Figure 33.1 The glutamine synthetase reaction.

deviation of the hands, anteverted nostrils and thin lips. Both initial patients had a flat or broad nasal roots and low set ears.

Patient two developed an erythematous rash at two weeks that became blistering after a few days (Figures 33.2–33.4). It became quite generalized. Patient three also developed necrolytic erythema (Figure 33.5). Histologic examination of the skin revealed intraepidermal blistering (Figure 33.6).

Electroencephalogram (EEG) in patient one showed very little cerebral activity and short bursts of theta waves and generalized seizures. One patient had a burst suppression pattern on EEG during sleep. Magnetic resonance imaging (MRI) of the brain of the first patient revealed delayed myelination, poor gyration and large subependymal cysts (Figure 33.7). In patient two there was also delayed myelination and poor gyration, as well as subependymal cysts (Figure 33.8).

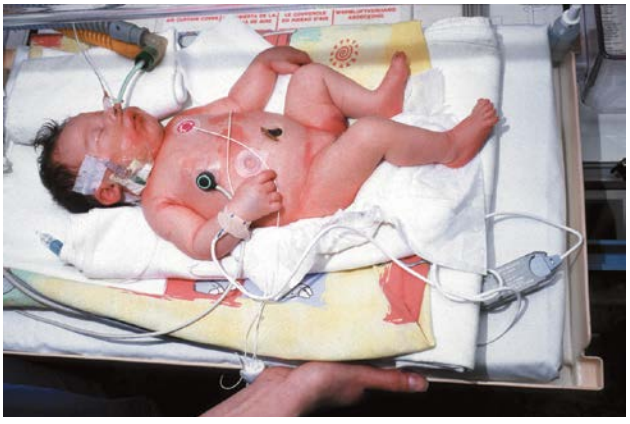

Figure 33.2 The necrolytic erythema of severe glutamine synthetase deficiency in the second patient described [1, 2]. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

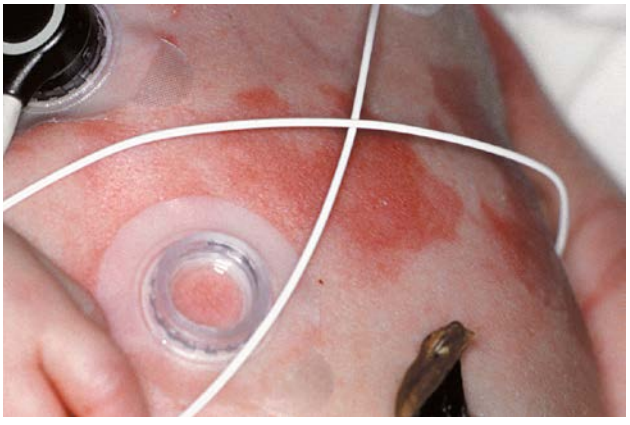

Figure 33.3 Closer view of the necrolytic erythema on the trunk of this patient. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

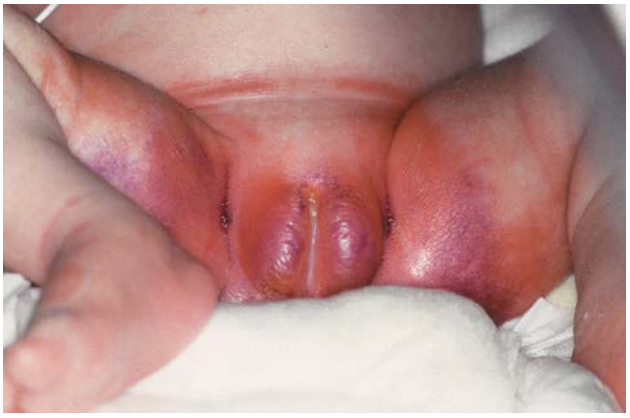

Figure 33.4 Necrolytic erythema in genital region in of the same patient. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

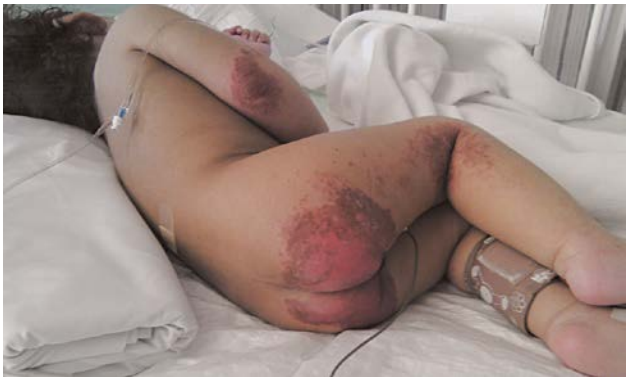

Figure 33.5 Necrolytic erythema in the third patient at 3 years of age. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

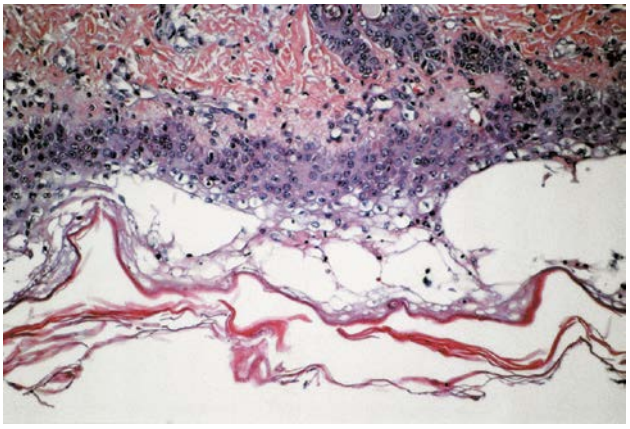

Figure 33.6 Histologic appearance of necrolytic erythema in severe glutamine synthetase deficiency. There was swelling of keratinocytes with condensed nuclei and intraepidermal blistering. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

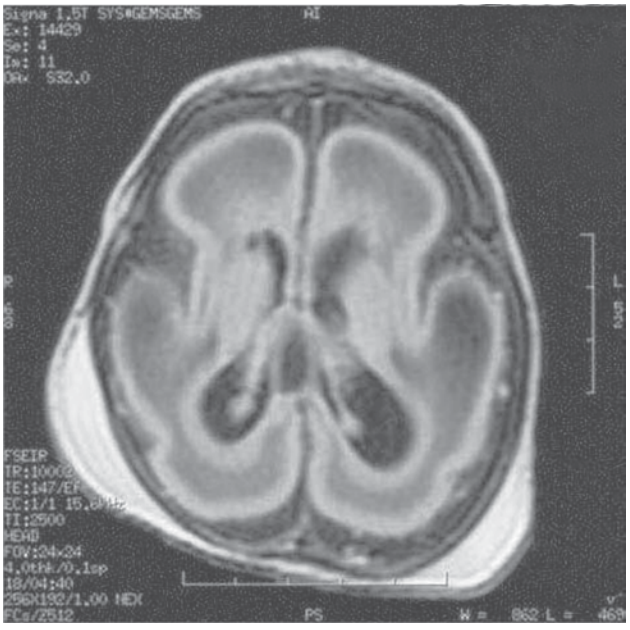

Figure 33.7 T_1-weighted MRI of the brain of the first patient illustrating severely delayed myelination and gyration and large subependymal cysts. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

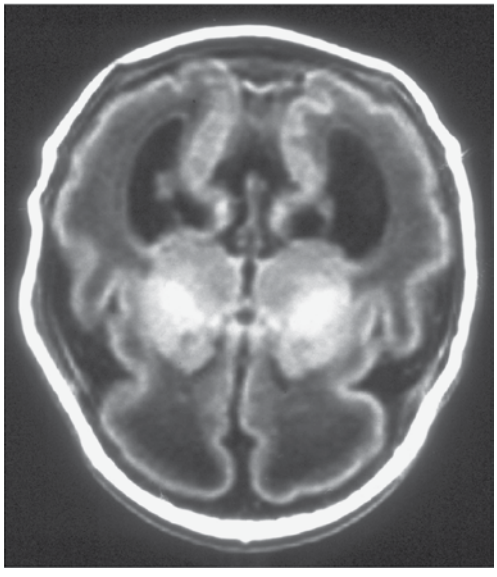

Figure 33.8 T_1-weighted MRI of the brain of patient two illustrating delayed myelination and gyration, and subependymal cysts. Illustration was kindly provided by Dr. Johannes Häberle, Universitats-Kinderspitall, Zurich.

Patients with this disease have moderate hyperammonemia. Levels ranged from 140 to 400 µmol/L (normal <110). Concentrations of glutamine in plasma were low in all three patients. Levels of 2 and 6 µmol/L were recoded (normal 300–800) [1]. In urine concentrations ranged from undetectable to 8 µmol/g creatinine (normal 52–3230). Concentrations in the CSF were 11 and 12 µmol/L (normal 352–1280).

GENETICS AND PATHOGENESIS

The disease is autosomal recessive. So far, each of the families have been consanguineous, the first two Turkish and the third Arabs from Sudan.

The gene *GLUL*, which codes for glutamine synthetase (glutamate-ammonia ligase), consists of 6 exons [1] over 1122bp and encodes a 374 amino acid polypeptide with a mass of 42kD [1, 4] the enzyme is mitochondrial [5]. The gene has been mapped to chromosome 1, 1q31 [6, 7]. There is a pseudogene on chromosome 9.

The mutations discovered to date represent a small area of exon 6, and all three involve arginine; two of them R324 and the other R341. In two, the arginine was converted to cysteine, and the other R324 was converted to serine. The R324 is at the ATP binding site, and the R41 is close to the glutamate binding site.

Glutamine synthesis is involved in ammonia utilization, nitrogen flux, and the regulation of acid-base balance. The enzyme is abundantly active in brain, liver, and muscle [8, 9]. Fetal requirements for glutamine are quite high [10]. In the brain, glutamine synthetase is found predominantly in astrocytes [11]. In the nervous system, glutamine is thought to be neuroprotective through lowering levels of both glutamate which is excitotoxic and ammonia. Glutamine is a substitute for the synthesis of NAD+ from deamino NAD.

TREATMENT

Treatment has been supportive. Seizures require anti-convulsant therapy. Treatment with glutamine in patient three led to increase in glutamine levels, but not to normal, and the burst suppression pattern of EEG improved.

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PART 4

DISORDERS OF FATTY ACID OXIDATION

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34

Introduction to disorders of fatty acid oxidation

INTRODUCTION

The genetically determined disorders of fatty acid oxidation represent a recently rapidly growing group of inborn errors of metabolism. The field, as we know it today, really dates from the discovery in 1982 of medium-chain acyl CoA dehydrogenase (MCAD) deficiency (Chapter 39) [1, 2]. Myopathic carnitine palmitoyl transferase (CPT II) (Chapter 38) deficiency was known for some time earlier, but considered among myopathies not a forerunner of expansive growth of knowledge, and HMG CoA lyase deficiency (Chapter 46) had been described but considered to be an organic acidemia. Multiple acyl CoA dehydrogenase deficiency (Chapter 45) was also known since 1976. The fact that MCAD deficiency turned out to be common, and largely the consequence of a single mutation, has contributed to the current recognition of the importance of this group of disorders. In subsequent years, the rates of discovery of previously unrecognized disorders of fatty acid oxidation was exponential. The advent of diagnosis by tandem mass spectrometry and its application to programs of expanded screening of newborns [3] have opened up this entire population to the prevention of death and disability.

A summation of the various pathways involved in fatty acid oxidation and their interrelations is shown in Figure 34.1. Abnormality in those pathways has often first been suggested chemically by the excretion of dicarboxylic acids in the urine. Dicarboxylic aciduria may also be dietary, especially in infants receiving formulas containing medium-chain triglycerides. When ß-oxidation is defective, ω-oxidation and hydroxylation take place in the microsomal P450 system. This takes place efficiently in the case of long-chain fatty acyl CoA compounds, but the affinity of the system for medium-chain chain compounds is so low that they are thought to result from ß-oxidation in peroxisomes of longer chain dicarboxylic or hydroxy acids [4].

Disorders of fatty acid oxidation may present with myopathy or cardiomyopathy. They may also present with sudden infant death syndrome (SIDS), but often the initial presentation is with a Reye-like episode of hypoketotic hypoglycemia, often with elevated blood concentrations of creatine kinase (CK), and uric acid, as well as transaminases [5]. Thus, in a hypoglycemic infant or child, evaluation of uric acid and CK (neither of which are routinely included in metabolic clinical chemistry panels in children's hospitals), serves as an alerting signal to the presence of a disorder of fatty acid oxidation. In a hypoglycemic patient, the absence of ketones in the urine signifies that it is hypoketotic. However, the presence of ketones in the urine may be misleading. Blood levels of free fatty acids and 3-hydroxybutyrate may be required to make this distinction. Hyperammonemia may be seen at the time of the acute episode, and this may lead to a diagnosis of Reye syndrome. Actually, most patients that we see today with Reye syndrome have a disorder of fatty acid oxidation, even if a liver biopsy appearance of microvesicular fat appears typical of Reye syndrome. A few such patients have a urea cycle defect, but we have seen orotic aciduria, the hallmark of ornithine transcarbamylase deficiency (Chapter 26) in a patient with MCAD deficiency.

We have developed a systematic algorithmic approach to the work up of a patient with a disorder of fatty acid oxidation (Figure 34.2). The patient is often referred after the initial episode has been treated with glucose and fluids, and examination of the urine is negative except in the case of HMG CoA lyase deficiency. Modern workup begins with the assay of the DNA for the A985G mutation in the *MCAD* gene or tandem mass spectrometry for acylcarnitines, or both. Study of blood and urine concentrations of carnitine and its ester fraction may point to the answer. In some patients, a controlled but prolonged fast is necessary to elucidate the nature of the defect, but this is less true since the availability of acylcarnitine profiles and mutational analysis for the MCAD mutations.

The normal response to fasting and the oxidation of fat begins with lipolysis, which releases free-fatty acids. In patients with disorders of fatty acid oxidation, concentrations of free-fatty acids are usually higher than those of 3-hydroxybutyrate in blood at times of illness and metabolic stress. Thus, assessment of the concentrations of free fatty acids and 3-hydroxybutyrate

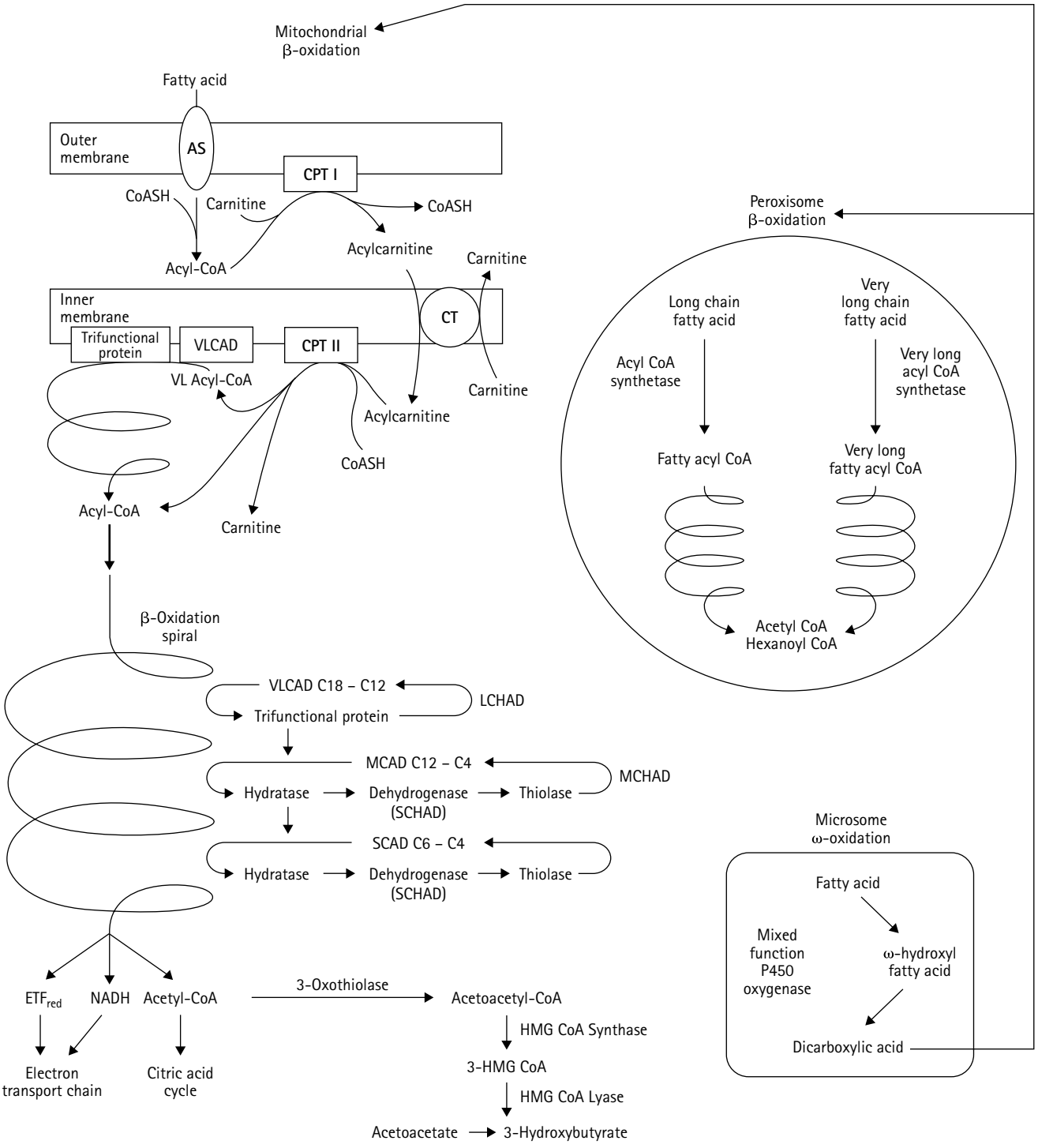

Figure 34.1 Metabolic pathways of fatty acid oxidation. Mitochondrial activities are central, but peroxisomal and microsomal oxidation also play a role.

in the blood is essential to the diagnosis of hypoketosis. Because fatty acids that accumulate in the presence of defective oxidation undergo ω-oxidation to dicarboxylic acids, a disproportionate ratio of dicarboxylic acids to 3-hydroxybutyrate in the organic acid analysis of the urine also indicates disordered fatty acid oxidation. Transport of long-chain fatty acids into the mitochondria, where ß-oxidation takes place, requires carnitine, and the entry of carnitine into cells such as muscle requires a specific transporter, which may be deficient in an inborn error of metabolism [6] (Chapter 35). Esterification of carnitine with fatty acyl CoA ester is catalyzed by acyltransferases, such as carnitine palmitoyl transferase (CPT) I (Chapter 37). The transport of the acylcarnitine across the mitochondrial

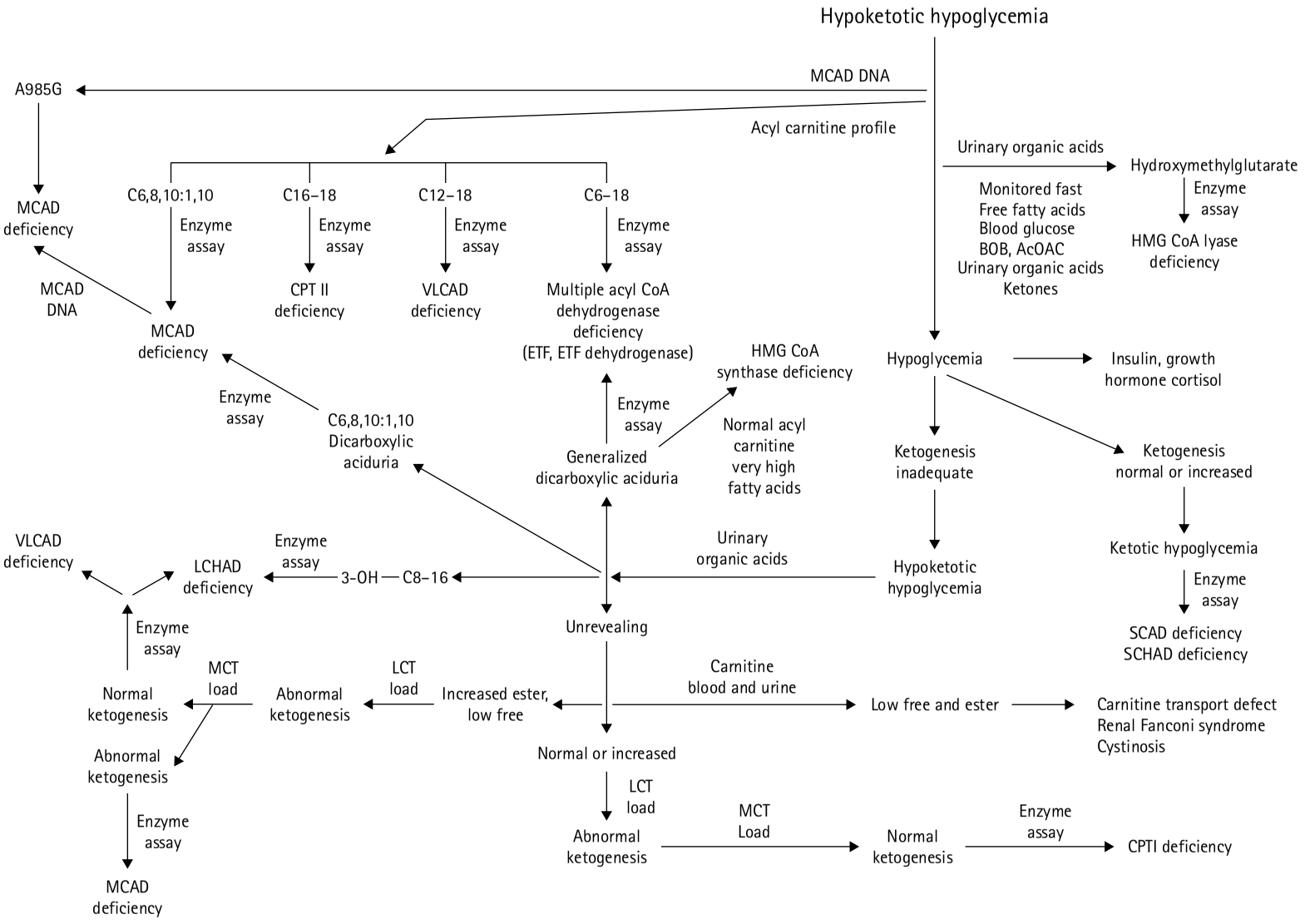

Figure 34.2 Algorithmic approach to the elucidation of hypoketotic hypoglycemia. Abbreviations employed: MCAD medium-chain acylCoA dehydrogenase; DNA deoxynucleic acid; CPT carnitine palmitoyltransferase; VLCAD very long-chain acylCoA dehydrogenase; ETF electron transfer flavoprotein; BOB 3-hydroxybutyric acid; AcOAc acetoacetic acid; HMG 3-hydroxy-3methylglutaric acid; SCAD short-chain acylCoA dehydrogenase; SCHAD short-chain hydroxyacylCoA dehydrogenase; MCT medium-chain triglycerides; LCT long-chain triglycerides; LCHAD long-chain hydroxyacylCoA dehydrogenase.

membrane is catalyzed by carnitine translocase (Chapter 36); and then hydrolysis, releasing carnitine and the fatty acylCoA, is catalyzed by a second acyltransferase, CPT II (Chapters 37 and 38). Inborn errors are known for each of these three enzymatic steps. In ß-oxidation, the fatty acid is successively shortened by two carbons, releasing acetylCoA.

Specific acylCoA dehydrogenases (ACADs) with overlapping specificities for chain length include: short-chain acyl CoA dehydrogenase (SCAD) (Chapter 42), medium-chain acyl CoA dehydrogenase (MCAD) (Chapter 39), and very long-chain acyl CoA dehydrogenase (VLCAD) (Chapter 40). In addition, a tri-functional enzyme catalyzes 3-hydroxyacyl dehydrogenation, 2-enoyl-CoA hydration, and 3-oxoacylCoA thiolysis [7]. Long-chain hydroxyacyl CoA dehydrogenase (LCHAD) is now known to be one of these three enzymatic steps of the tri-functional protein (Chapter 41).

Diseases involving defects in each of these steps have been defined. Among these diseases, and in five others that have been identified, only MCAD deficiency is easy to diagnose definitively. This is because most MCAD deficient patients, defined prior to expanded newborn screening, have had the same mutation, an A→G change at nucleotide 985, which is readily assessed after amplification of the DNA by the polymerase chain reaction. Even in this disease, however, at least five infrequent mutations are known [8], and at least one common mutation T199C is found in patients identified by newborn screening. Acylcarnitine profiling should lead to the diagnosis in each of these disorders [9] (Table 34.1), but experience is not yet available to indicate how often this approach might miss the diagnosis in an established patient who has become carnitine depleted. Acylcarnitine profiling has also been employed in the analysis of postmortem bile to identify patients with disorders of fatty acid oxidation initially thought to have sudden infant death syndrome [10].

During the long fast, patients must be monitored closely so that symptomatic hypoglycemia is avoided. Testing is best done in units where the staff has experience with the protocol. An intravenous (IV) line is placed to ensure access for therapeutic glucose, and bedside monitoring of blood concentrations of glucose is done at regular intervals. In abnormalities of fatty acid oxidation, fasting must be long enough to exhaust stores of glycogen and require the mobilization of fat and its oxidation. This usually requires 17–24 hours.

The specific enzyme assays for specific disorders are technically demanding and not generally available. A good next step following the fast, if a specific disease is not identified, is to pursue a more general study of metabolism in cultured cells [11, 12] in which CoA or carnitine esters are separated and identified by HPLC after interaction with ^{14}C or ^{13}C-labeled hexadecanoate.

Experience with the diagnosis and management of disorders of fatty acid oxidation was summarized in 1999 for a series of 107 patients with a spectrum of disorders [13]. The severity of these diseases is indicated by the fact that only 57 of the 107 patients were alive at report. An additional 47 siblings had died in infancy for a total of 97 deaths, 30 percent in the first week of life and 69 percent before one year. These data symbolize the importance of newborn screening in prevention, because the avoidance of fasting would prevent many deaths. Seventy-three percent were judged to have hepatic clinical presentations, which included hypoketotic hypoglycemia, hepatomegaly, Reye syndrome, and microscopic hepatic steatosis. True hepatic failure was seen in 11 percent and occurred only in carnitine translocase deficiency and multiple acyl CoA dehydrogenase deficiency; a single patient with LCHAD deficiency had cholestasis. In addition, a previously unrecognized defect in the transport of long-chain fatty acids has been reported to cause acute liver failure and hypoketotic hypoglycemia [14]. Oxidation of C14-18 fatty acids by fibroblasts was defective. The clue to the diagnosis was low concentrations of C14-18 fatty acids and elevated carnitine, a very unusual pattern, in biopsied liver. Cardiac presentations were seen in 51 percent of patients. There were arrhythmias as well as cardiomyopathy. Skeletal muscle involvement in 51 percent of patients included myalgia, myopathy, and rhabdomyolysis with myoglobinuria.

Treatment of disorders of fatty acid oxidation [15] can be considered under two headings: acute management of acute metabolic imbalance, and chronic preventive therapy.

Acute management rests on the provision of a plentiful supply of glucose. This is designed to treat hypoglycemia. It is also designed to inhibit lipolysis. So, IV solutions of 10 percent glucose or more are the rule even in those who are normoglycemic, for instance, a patient with rhabdomyolysis. Insulin, along with glucose, may be necessary to maintain normoglycemia, and a central line or portacath may be required. Carnitine is given, preferably IV, in doses of 100–300 mg/kg, because of greater bioavailability and the avoidance of diarrhea resulting from large oral doses. Carnitine therapy is mandatory in carnitine transporter deficiency. It has been controversial in long-chain fatty acid disorders because of a theoretical risk that accumulation of long-chain acyl carnitines may be arrhythmogenic, as found in experimental situations [16]. Clinical experience is not consistent with this danger, and most support the administration of carnitine, not only to treat the deficiency of free carnitine that develops, but also to promote the detoxifying excretion of accumulated CoA esters as carnitine esters and the restoration of supplies of CoA [17].

The mainstay of long-term management is the avoidance of fasting. Our patients are supplied with letters indicating the need for parenteral glucose whenever intercurrent illness or vomiting preclude the enteral route. We recommend that IV glucose be given in this situation even if the blood concentration of glucose is normal on arrival at the emergency room. Overnight fasting is minimized by the use of oral corn starch (1 g/kg hour; 8 g/tbsp). In some situations, continuous nocturnal intragastric feeding has been employed, but we do not recommend it. Restriction of long-chain dietary fat is generally prudent as is long-term oral carnitine. Medium-chain triglyceride supplementation is therapeutic in long-chain fatty acid defects.

Table 34.1 Acylcarnitine profiles of plasma in the diagnosis of disorders of fatty acid oxidation

Disorder	Acylcarnitine	Control reference* (μmol/L)	Patient range (μol/L)	Organic acid analysis
MCAD	C6	0.12	0.12–2.14	Hexanoyl-, suberyl-, phenylpropionyl-, glycine.
	C8	0.22	1.28–12.24	
	C8/C8:1	2.32	6.49–46.49	
	C10:1	0.22	0.26–1.84	Dicarboxylic aciduria
LCHAD	C16/C8:1	2.89	10.8–258.96	3-OH-Dicarboxylic, dicarboxylic aciduria
	C16OH	0.02	0.12–0.60	
	C18:1OH	0.01	0.14–0.86	
VLCAD	C14:1	0.18	0.76–13.28	Dicarboxylic aciduria
	C14:1/C8:1	1.48	8.26–427.05	
	C14:2	0.08	0.30–3.48	
SCAD/EMA	C4/C3	0.98	0.71–9.0	Ethylmalonic, methyl-succinic aciduria, butyrylglycine
	C4	0.32	0.62–1.28	
	C5/C3	0.80	0.19–4.52	
	C5	0.22	0.16–0.64	
CPT I**	C0/C16+18	2–32	63–291	
CPT II	C14:1/C8:1	1.48	2.50–42.79	
	C16/C8:1	2.89	101.24–221.65	
	C16	0.24	2.06–3.94	
	C16:1	0.08	0.50–0.86	
	C18	0.10	0.64–1.411	
	C16+C18/C2	0.011–0.095	0.08–0.56	
Multiple AcylCoA-Dehydrogenase	C4/C3	0.98	0.70–8.19	Isobutyryl-, isovaleryl-, 2-methyl-butyryl-glycine, glutaric aciduria, dicarboxylic aciduria
	C5/C3	0.80	1.39–2.01	
	C6	0.12	0.04–3.54	
	C5DC	0.06	0.04–0.08	
Carnitine Transporter	C0		↓	
	Cesters		↓	
Carnitine Translocase	C16	2.06–3.94	8.85	
	C18	0.64–1.44	↑	
HMG CoA lyase	C5OH	1.06	0.08–1.42	
Methyl glutaryl		0.02	0.08–0.62	

Adapted from Vreken et al. [9] and other sources.

* 95th percentile of the reference range except where a range is given. Abbreviations include EMA, ethylmalonic acidemia, CPT, carnitine palmitoyl transferase; DC- dicarboxylic acid.

** Absence of long chain acyl carnitines.

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35

Carnitine transporter deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, seizures, vomiting, lethargy progressive to coma; cardiomyopathy; chronic muscle weakness; carnitine deficiency in plasma and muscle, and increased excretion of free carnitine in urine; defective transport of carnitine into cultured fibroblasts and mutations in the *SLC22A5* gene which codes for the sodium ion-dependent carnitine transporter OCTN2.

INTRODUCTION

The inborn errors of fatty acid oxidation, including carnitine transporter deficiency (CTD) [1, 2], represent a relatively recently recognized area of human disease. The rate of discovery of distinct disorders has increased rapidly since the discovery of medium-chain acyl CoA dehydrogenase (MCAD) deficiency in 1982 (Chapter 39). Deficiency of carnitine is common in these disorders in which fatty acyl CoA compounds accumulate which then form esters with carnitine and are preferentially excreted in the urine. Carnitine deficiency may also be profound in organic acidemias, such as propionic acidemia, for the same reason. The transport of carnitine into fibroblasts is inhibited by long- and medium-chain acylcarnitines [3], and this may be an additional factor in carnitine deficiency in disorders of fatty acid oxidation. Primary carnitine deficiency resulting from an abnormality in the synthesis of carnitine from protein-bound lysine has not yet been observed. Many of the patients reported early as primary carnitine deficiency have turned out to have MCAD deficiency. Deficiency of carnitine as a result of abnormality in the transporter (Figure 35.1) that facilitates its entry into certain cells has been referred to as primary carnitine deficiency [1]. The carnitine transporter is an organic cation transporter (OCTN2) in the solute carrier family. The gene *SLC22A5* has been cloned, and increasing numbers of mutations are being found [4–6].

CLINICAL ABNORMALITIES

The classic, and frequently the initial presentation of CTD, is hypoketotic hypoglycemia, as in most disorders of fatty acid oxidation. The first patient reported (Figure 35.2) [1] presented at three months, comatose, limp, and unresponsive in the afternoon after a prolonged overnight fast. She was acidotic; the serum bicarbonate was 16 mmol/L and the arterial pH 7.17. The blood concentration of glucose was 0.39 mmol/L (7 mg/dL) and that of the cerebrospinal fluid (CSF) was 0.2 mmol/L (4 mg/dL). Resuscitation required intubation, assisted ventilation, and parenteral glucose and saline. Acute episodes of hypoketotic hypoglycemia are potentially fatal (Figure 35.3) [7] and may be sudden and unexpected. An infant of a vegetarian mother died aged five days [8]. Episodes usually occur before two years of age and follow fasting [3, 9].

Modest hepatomegaly is characteristic of this condition. Biopsy of the liver shows microvesicular lipid [10], a finding, like the rest of this clinical picture, that might lead to a diagnosis of Reye syndrome.

Clinical chemistry in the acute hypoketotic episode is also consistent with Reye syndrome, with hyperammonemia and increased levels of transaminases. The initial patient had a level ammonia of 338 mmol/L, slightly prolonged prothrombin time, an aspartate transaminase (AST) of 248 and alanine transaminase (ALT) of 149 IU/L [1]. Recurrent

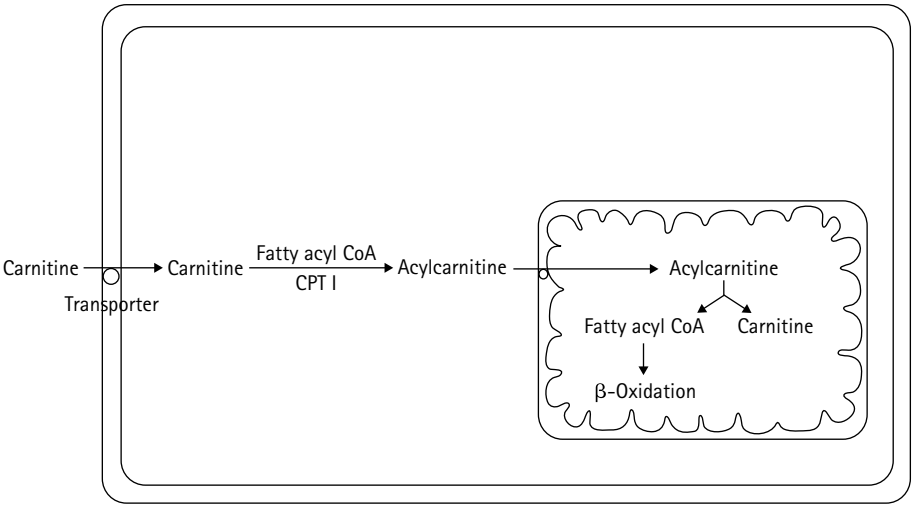

Figure 35.1 The carnitine transporter and its role in fatty acid metabolism.

Figure 35.2 A 17-month-old patient who was the initial reported patient [1] with transporter defect. She presented at three months with hypoglycemic coma precipitated by an intercurrent illness and prolonged fasting. This episode left her with severe brain damage reflected in her vacant expression. She died shortly after the picture was taken because of complications of a gastrostomy. (Illustration was kindly provided by Dr Charles Stanley of the Children's Hospital of Philadelphia.)

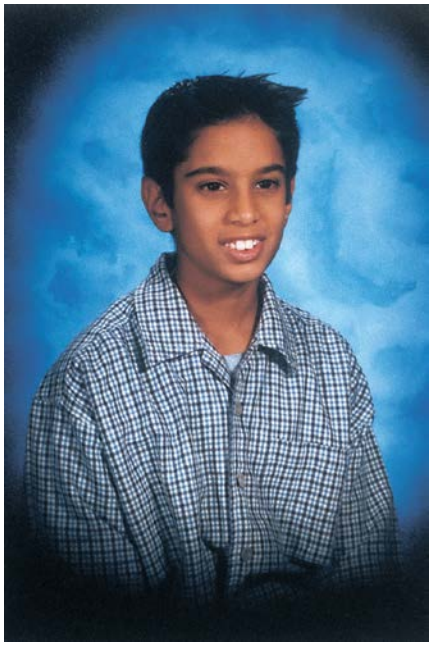

Figure 35.3 JS: A 12-year-old boy with carnitine transporter deficiency. The disease is exquisitely responsive to carnitine. His death at 13 years highlights the dangerous nature of the disease and the importance of close follow up of carnitine status and expert management.

episodes of Reye syndrome have been reported. Uric acid concentrations are also elevated at the time of the episode. This, along with the elevation of creatine phosphokinase (CK) [3] should strongly suggest a disorder of fatty acid oxidation.

Examination of the urine may reveal no ketonuria [1, 3], and this should strongly suggest the diagnosis. However, in the height of the hypoglycemic illness, there may be misleading ketonuria in any disorder of fatty acid oxidation. Quantification of the plasma concentration of 3-hydroxybutyric acid or of acetoacetic and 3-hydroxybutyric acids at this time will provide definitive evidence of impaired ketogenesis, but this information is not usually available to the clinician. Dicarboxylic aciduria is usually notably absent [1, 7].

Cardiomyopathy is the other classic way in which this disorder presents [3, 7, 10] and may be expected in any patient not given the benefit of diagnosis and treatment with carnitine. It was the most common presenting complaint in

15 patients [6] and present in 100 percent of 20 early reported patients. The patient of Waber and colleagues [10] reported with progressive cardiomyopathy and cardiac failure successfully treated with carnitine was subsequently shown to have the transporter defect. A median age of onset of cardiac symptoms was three years [3]. Cardiomyopathy and congestive cardiac failure had begun at seven years of age. Onset may be with rapidly progressive heart failure [3] or a murmur, and cardiomegaly may be found on routine physical examination or examination at the time of hypoglycemia. Roentgenograms and echocardiography reveal cardiac enlargement and increased thickness of the left ventricular wall. Electrocardiogram (ECG) reveals left ventricular hypertrophy. Nevertheless, the cardiomyopathy has also been described as characteristically dilated [11], and this has repeatedly been confirmed by cardiac catheterization. This may be expected to be a lethal disease in which patients without carnitine supplementation display cardiac failure progressive to death. Cardiac disease may also present with arrhythmia. Death in a sibling has been recorded in at least eight families [3]. A 12-year-old boy who died suddenly of cardiomyopathy following a routine surgical procedure was found to have a low concentration of carnitine in plasma and defective transport of carnitine in fibroblasts [12].

Muscle weakness or hypotonia is the third major manifestation of disease. It may be present along with other features, particularly those of the heart, but in two patients it was the only manifestation [3]. The picture may be that of a progressive proximal myopathy [11]. Biopsy of muscle reveals lipid storage myopathy [1].

An unusual presentation was that of an infant with profound peripheral neuropathy [13]; she had absent deep tendon reflexes and could not walk. Electromyogram (EMG) was consistent with sensory and motor neuropathy, and muscle biopsy reported neurogenic atrophy. Delay in diagnosis has been another characteristic of this disease. In nine patients, the delay was between one and six years after the onset of symptoms [3], and in this time, all developed cardiomyopathy, and all but one had muscle weakness. In some patients, mild muscle weakness may not have been noted because of the attention devoted to the major cardiac manifestations.

The advent of newborn screening for this disease made it clear that it can also be asymptomatic well into adult life [14–16]. In programs of newborn screening using tandem mass spectrometry (MS/MS), it has become a common occurrence to find that a positive newborn screen for C_0-deficiency results from an asymptomatic but affected mother. Confronted with an abnormal C_0-screen, we all now study the mother as well as the baby. In 15 reported families in which the defective maternal transporter was confirmed by studies of uptake of carnitine by fibroblasts, mutational analysis, or both, the infant was normal in all but one; in that family, mother and infant were affected. Most mothers were asymptomatic. Three had low stamina, easy fatigability with exercise and fasting intolerance [16]. Echocardiograms in four were normal [14].

Asymptomatic status has been observed in a father and son with defective fibroblast uptake of carnitine and the same mutations as another who presented in infancy with a Reye-like syndrome of hepatic disease and encephalopathy [17]. Lack of correlation between levels of carnitine and phenotypic severity is consistent with the unpredictable role of intercurrent infection [18].

MS/MS has made screening for this disease an integral part of most programs of newborn screening [19, 20].

GENETICS AND PATHOGENESIS

Transmission of the disorder is autosomal recessive. Affected siblings of both sexes have been observed, and consanguinity has been present in at least five families [3, 21]. Prevalence is not yet known, but there were ten patients in the series of 107 with disorders of fatty acid oxidation in the experience of Saudubray *et al.* [11] in Paris, and newborn screening experience should soon give reliable prevalence data. Among 313 patients with an autopsy diagnosis of sudden infant death syndrome (SIDS), three were designated as transporter defects on the basis of hepatic steatosis and very low hepatic carnitine along with low esterified carnitine [22].

Prior to newborn screening, the diagnosis was usually suspected on the basis of a low concentration of free carnitine in plasma. In the first patient, the total plasma carnitine ranged from 0 to 2.2 mmol/L and no free carnitine could be detected. In 20 patients [3], total plasma carnitine ranged from 0 to 9 mmol/L, with 18 having values less than 4.2 mmol/L. In controls, total carnitine was 40–60 mmol/L (Table 33.1). The acylcarnitine profile reveals a decrease in free and esterified carnitines.

Concentrations of carnitine in muscle are also quite low. In 13 patients studied, the range for total carnitine was from 0.05 to 17 percent of the normal mean. In the liver, the total was 5 percent of normal. The excretion of carnitine in the urine is inappropriately high, consistent with defective renal tubular reabsorption [10]. At a time when the plasma carnitine approximated zero, the renal excretion was 126 mmol/g creatinine (normal, 167–425) [1], and following a dose of 100 mg/kg of oral carnitine, the plasma carnitine rose only to 21 mmol/L, but urinary excretion increased to 2911 mmol/g creatinine. After four months of carnitine treatment, the plasma concentrations reached the low normal range, while urinary excretion was four to five times normal. The fractional excretion rate for free-carnitine was nearly 100 percent of the filtered load. On withdrawal of treatment, the fractional excretion exceeded the filtered load.

The nature of the defect has been demonstrated by study of the uptake of carnitine *in vitro* by cultured fibroblasts (see Figure 35.3) [1, 3]. In control cells, the uptake of ^{14}C-labeled carnitine was via a high-affinity, carrier-mediated transport process with an apparent Km of 3.24 ± 0.5 and a V_{max} of 1.67 ± 0.19 [1] consistent with previous reports [23]. Fibroblasts from patients have shown

little uptake of carnitine; at a concentration of carnitine of 5 mmol/L, control uptake was 0.94 and a patient uptake was 0.1 pmol/min per mg protein [1]. High-affinity transport is best shown at lower concentrations; up to 1 mmol/L uptake was negligible. Uptake in patients at high concentrations, such as 10 or 20 mmol/L reflect a second low-affinity transporter [22] or passive diffusion [24]. Transport of carnitine in control fibroblasts is sodium-dependent [1]. The uptake of carnitine by fibroblasts at 5 mmol/L showed no overlap among patients and controls. The velocity of carnitine uptake can be measured in lymphoblasts, as well as fibroblasts [25]. Patients display rates below 10 percent of control. Heterozygosity can be demonstrated in some patients by rates below 40 percent of control. Low uptake of carnitine has also been demonstrated in cultured myocytes derived from patients [26]. Prenatal diagnosis has been accomplished by demonstration of defective uptake of carnitine from amniocytes of an affected fetus [27].

In response to the administration of carnitine, levels in the liver return to normal, while those in muscle respond poorly, indicating that the transport defect includes muscle and kidney, but not the liver. Consistent with this, the low Km and preference for L-isomer that characterize the uptake of carnitine by fibroblasts is shared by heart and muscle [23, 24], but not by the liver [28].

The gene for the carnitine transporter, *SLC22A5*, has been mapped to chromosome 5q31.1 the locus for CTD in a large Japanese kindred [29]. The gene has ten exons over 26 kb. It codes for the organic cation transporter OCTN2, which is one of a family of organic cation (OCTN) sodium ion-dependent transporters [30]. The protein contains 557 amino acids and has the properties of a high-affinity transporter. Many mutations have now been identified in patients with this disease [3, 31–38]. Most individual families have had unique mutations. There is an OCTN2 database (www.arup.utah.edu/database/OCTN/OCTN2). There have been a few instances of the same mutation in unrelated patients [5, 32–36]. A few stop codons and frameshifts have been defined [3, 30]. A lack of correlation between genotype and phenotype has been discussed [3, 36]. However, decisions as to severity of phenotype often rest on whether or not hypoglycemia once occurred early in life. Differences in presentation could simply reflect the chance occurrence of an intercurrent illness that led to fasting.

Among ethnic differences, an 11-bp deletion was found in unrelated patients from Switzerland and neighboring northern Italy [3] and R169W was found in two unrelated families in Italy [36]. In Japan, where the disease appears relatively frequent, most families have had a few mutations [39]. In a survey of 973 unrelated Japanese [39], 14 were found to have low levels of carnitine, and of these, six had mutations in the gene for OCTN2: W132X, S467C, W283C, and M179L. These data gave a carrier frequency of 1 percent in Japan. Echocardiographic study indicated asymptomatic cardiac hypertrophy in these heterozygotes. The R254X nonsense mutation on exon 4 may be prevalent in the Lebanese [40]. Two Iranian Jewish siblings with the same mutation (R399Q) had very different clinical presentations [41]. One presented in coma at two years of age following gastroenteritis. Her older sibling had proximal limb girdle weakness, which was markedly improved following treatment with carnitine. In studies with confocal microscopy, some mutant OCTN2 matured normally to the plasma membrane, while others were retained in the cytoplasm [42]. A database [43] has summarized 49 different mutations; most were private.

An animal model of the carnitine transporter defect, the juvenile visceral steatosis (jvs) mouse [44], has autosomal recessive fatty infiltration of the liver, hypoglycemia, and hyperammonemia two weeks after birth, and very low levels of carnitine in blood and muscle, along with defective renal reabsorption of free carnitine. The hyperammonemia results from decreased expression of genes for enzymes of the urea cycle; low levels of mRNA are associated with low levels of all of the hepatic enzymes of the urea cycle [45]. Treatment with carnitine corrects the abnormal expression and urea cycle enzyme activity [46]. The jvs gene has been mapped to mouse chromosome 11, which is syntenic with the SLC22A5 locus on human chromosome 5 [47].

Analysis of the organic acids of the urine of these patients is usually normal. The absence of dicarboxylic aciduria, especially at times of acute illness and hypoglycemia, contrasts sharply with findings in patients with defects in β-oxidation, such as MCAD deficiency. Patients with deficiency of carnitine palmitoyl-transferase I (Chapter 37) also develop hypoketotic hypoglycemia without dicarboxylic aciduria [48]. Comparisons of alterations of plasma carnitine in various disorders are shown in Table 35.1. Low free and total carnitine in plasma along with urinary free carnitine that is paradoxically maintained is suggestive of a transporter defect.

The response to fasting in a patient with defective carnitine transporter showed hypoketosis throughout and hypoglycemia by 12 hours (Figure 35.4) [1]. The fast was stopped when the plasma glucose reached 2.8 mmol/L (51 mg/dL), at which time, the patient remained asymptomatic. Levels of free-fatty acids in plasma rose sharply to 2.22 mmol/L, but the level of 3-hydroxybutyrate remained flat at 0.27 mmol/L. Blood concentrations of ammonia rose. Treatment with carnitine corrected this patient's impaired hepatic oxidation of fatty acids; and she was able to fast for 24 hours without hypoglycemia. Levels of 3-hydroxybutyrate rose to 2 mmol/L, higher than the free-fatty acids (1.25 mmol/L).

Diet may contribute to the pathogenesis of symptoms in this disease. A 12-year-old patient who died suddenly following surgery [13] had been exposed to an essentially vegetarian diet for some time. The three-month-old initial patient [1] had been changed from a cow's milk protein containing formulation to a soy protein preparation that contained no carnitine, four weeks prior to the episode of hypoketotic hypoglycemia.

Table 35.1 Differential diagnosis of disorders involving carnitine

	Plasma total (mmol/L)	Carnitine esterified (% of total)	Urinary carnitine
Control	40–60	30	Normal
Carnitine transporter deficiency	5	30	Paradoxically high free
Carnitine palmitoyl transferase (CPT) I deficiency	60–100	20	Normal or high
Carnitine translocase deficiency	5–30	80–100	High ester
Carnitine palmitoyl transferase (CPT) II deficiency	10–40	40–80	Normal or high ester
Defects in β-oxidation	10–30	30–60	High ester
3-Hydroxy-3-methylglutaryl CoA lyase deficiency	10–30	30–60	High ester

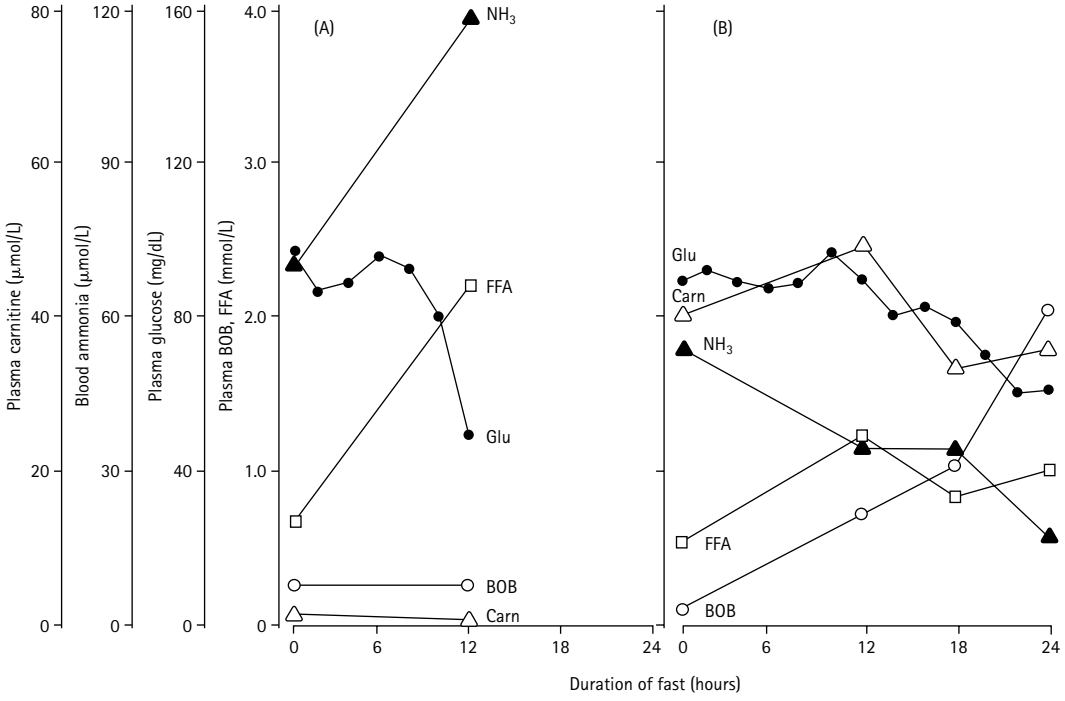

Figure 35.4 The response to fasting in a patient with the carnitine transporter defect. (A) In the control state, hypoglycemia (glu) was prominent at 12 hours, and there was no evident ketogenesis (3-hydroxybutyrate (BOB)) despite elevation of free-fatty acids (FFA). (B) Following treatment with carnitine, fasting for 24 hours was without hypoglycemia, and ketogenesis was evident in the rising BOB. [1].) (Reprinted with permission from the *New England Journal of Medicine*.)

The pathogenesis of symptoms of hypoketotic hypoglycemia reflects the role of fat in energy metabolism. Hypoglycemia after short periods of fasting usually represent disorders of carbohydrate metabolism. The oxidation of fatty acids is not a major source of energy until relatively late in fasting. It usually takes 15–24 hours of fasting to induce hypoglycemia in a patient with a disorder of fatty acid oxidation. An individual who never fasted beyond 12 hours would usually be protected against this manifestation.

The metabolism of fat begins with lipolysis; those patients with defective fatty acid oxidation have high ratios of free fatty acids to 3-hydroxybutyrate in blood after fasting. Once transported into cells, carnitine is esterified with acyl CoA esters, including those of fatty acids resulting from lipolysis. The esterifications are catalyzed by carnitine acyl transferases, such as carnitine palmitoyl transferase (CPT) I. Carnitine translocase then catalyzes the transfer of the fatty acylcarnitines across the membrane into the mitochondrion, where hydrolysis to fatty acyl CoA and free or recycled carnitine is catalyzed by CPT II. Fatty acyl CoA compounds then undergo β-oxidation in which there is successive shortening by two carbon atoms releasing acetyl CoA. In muscle, this is largely oxidized via the citric acid cycle, while in the liver ketogenesis proceeds via the successive action of 3-hydroxymethylglutaryl (HMG) CoA synthase and lyase-yielding acetoacetate, which is converted to 3-hydroxybutyrate.

TREATMENT

Treatment of this disease with carnitine has been highly successful [3, 10]. Levels of free-carnitine in plasma and liver are readily restored, preventing further attacks of hypoketotic hypoglycemia. Developmental delay or seizures induced by hypoglycemic attacks prior to treatment persist, but are not progressive.

Cardiomyopathy and failure respond dramatically to treatment [9, 10] and early therapy with carnitine has been reported [49] to prevent cardiomyopathy. Heart size is reduced to normal within months. Doses have ranged from 50 to 150 mg/kg p.o. [40]. Doses as high as 400 mg/kg have been recommended [15]. Intestinal tolerance often mandates lower dosage. Skeletal muscle weakness improved, although mild proximal muscle weakness has occasionally persisted. However, muscle concentrations of carnitine were documented to increase only slightly to 22–80 mmol/g; control levels are 2500–3500 mmol/g. These observations suggested that muscle oxidation of fat and muscle function may be unaffected until the intracellular muscle concentration of carnitine falls below 30–50 mmol/L or 2–4 percent of normal. Biopsied muscle revealed a decrease of stored lipid with treatment, but not a disappearance [1].

The occurrence of asymptomatic mothers uncovered by programs of newborn screening raises questions as to treatment. It appears prudent to treat these women [15]. This may be particularly true during a subsequent pregnancy, as pregnancy lowers carnitine stores.

Studies which indicated failure of mutant transporter proteins to mature normally to the plasma membrane [42] led to studies of the effects of small molecules on carnitine transport *in vitro*. Phenylbutyrate, quinidine, and verapamil were found to stimulate transport raising the possibility of pharmacologic therapy.

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Carnitine-acylcarnitine translocase deficiency

MAJOR PHENOTYPIC EXPRESSION

Episodes of life-threatening illness with cardiac arrhythmia; coma with hypoketotic hypoglycemia, and hyperammonemia; sudden infant death; hepatomegaly weakness of muscle; deficiency of free carnitine and increased long-chain acylcarnitines; deficiency of carnitine translocase; and mutations in *SLC25A20*.

INTRODUCTION

Carnitine translocase (carnitine:acylcarnitine carrier) deficiency is a recently discovered disorder of fatty acid oxidation. First described in 1992 [1], the disease accounted for ten of 107 patients in the Saudubray experience with abnormalities in the oxidation of fatty acids [2]. Many patients have developed symptoms and died in infancy [1–4].

Mitochondrial oxidation of long-chain fatty acids provides an important source of energy for the heart, as well as for skeletal muscle during prolonged aerobic work and for hepatic ketogenesis during long-term fasting. The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to the enzymes of β-oxidation. The shuttle consists of three enzymes (carnitine palmitoyltransferase [CPT] 1, carnitine-acylcarnitine translocase [CACT], CPT 2) and a small, soluble molecule (carnitine) to transport fatty acids as their long-chain fatty acylcarnitine esters. Carnitine is provided in the diet (animal protein) and also synthesized at low rates from trimethyllysine residues generated during protein catabolism. Carnitine turnover rates (300–500 μmol/day) represent <1 percent of body stores; 98 percent of carnitine stores are intracellular (total carnitine levels are 40–50 μM in plasma versus 2–3 mM in tissue). Carnitine is removed by urinary excretion after reabsorption of 98 percent of the filtered load; the renal carnitine threshold determines plasma concentrations and total body carnitine stores [5].

Long-chain fatty acids must be esterified with carnitine before they can be transported into the mitochondria where β-oxidation takes place. The translocase, CACT, catalyzes the transfer of the acylcarnitines across the inner mitochondrial membrane (Figure 36.1). The enzyme is one of ten related membrane carrier proteins, carnitine cycle,

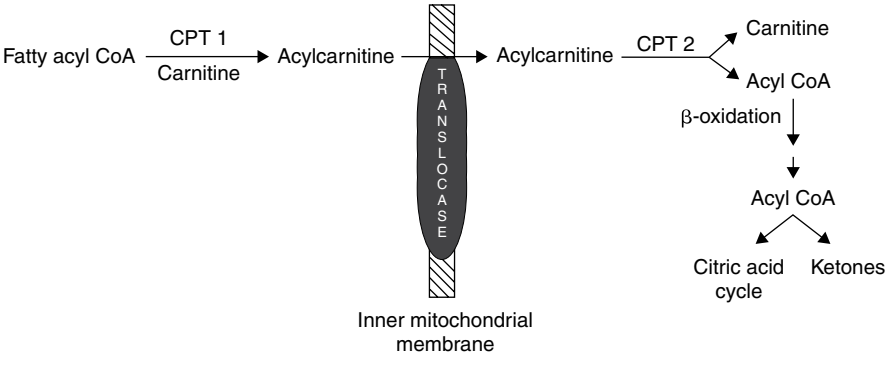

Figure 36.1 Long-chain fatty acid oxidation and the role of carnitine translocase. CPT, carnitine palmitoyltransferase.

that shuttle proteins from the cytosol to the mitochondrial matrix. Another is the ornithine transporter that is defective in the hyperornithinemia, hyperammonemia, homocitrullinuria (HHH) syndrome (Chapter 31). Once inside the mitochondrion, the acylcarnitine is split through the action of CPT 2 to free carnitine and the fatty acyl CoA ester, which is then substrate for β-oxidation. Each step in the sequence is essential if fat is to be burned as fuel or converted to ketones and used for gluconeogenesis. CACT and early-onset CPT 2 (Chapter 38) deficiencies have an extremely high neonatal mortality rate. Late-onset CPT 2 deficiency is characterized only by episodic rhabdomyolysis. CPT type 2A deficiency may often be benign, although early presentation with hypoketotic hypoglycemia certainly occurs [6].

The gene has been cloned and some mutations identified [7].

CLINICAL ABNORMALITIES

The hallmark of disorders of fatty acid oxidation is hypoketotic hypoglycemia (Figure 36.2), and this has often occurred in the neonatal period in this disease [4]. Hyperammonemia, encephalopathy, cardiomyopathy hepatopathy, and myopathy are typically seen in the neonatal period [8, 9]. The major characteristic of this disorder has been the occurrence of cardiac arrhythmias [1–4, 9].

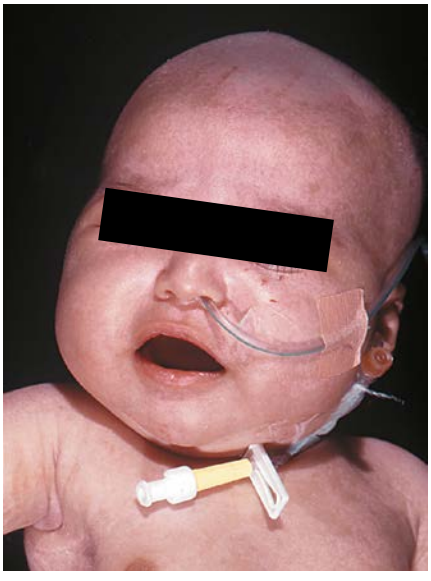

Figure 36.2 One-month-old Saudi female with CACT deficiency. She presented with severe hypoketotic hypoglycemia, hyperammonemia, and coma. She also had a cardiomyopathy that improved. She required peritoneal dialysis and ventilation. She was discharged home at three months of age in good condition [8]. A unique molecular defect (p.Q238R) was found; the family underwent preimplantation genetic diagnosis and had a normal child [7].

Episodes typically follow prolonged fasting, which is a common response of infants to intercurrent infectious disease. In one patient [3], a dextrostix reading of zero was recorded during an episode at 36 hours in which the infant was found to be pale, unresponsive, and hypothermic (34.5°C). Another patient [1] had a seizure, apnea, and bradycardia at 36 hours of age. This episode, which appeared to have been provoked by fasting, led to apnea requiring mechanical ventilation and hypotension, which was treated with lidocaine and dopamine. This patient went on to have repeated episodes of vomiting, lethargy, and coma following intercurrent illness and attendant fasting; each responded to the intravenous (IV) administration of glucose. Another patient [3] developed a second episode of hypoglycemia (0.7 mmol/L) on the third day of life, despite receiving 5 percent IV glucose; the test for ketones in the urine was negative. The hypoglycemia was corrected by increasing the rate of infusion of glucose, but the patient deteriorated clinically and died at eight days of age. Undetectable glucose was the case in a patient who presented at 36 hours [10].

A previous sibling of the first patient [1] died at four days of age of what might be interpreted as sudden infant death syndrome (SIDS). He had a sudden, unexplained cardiorespiratory arrest at two days and died two days later. The previous sibling of another patient [10] died of cardiorespiratory arrest at 24 hours. Two previous siblings of a patient with the disease, who died suddenly at 12 months, had died in the first 12 months [11].

Cardiomyopathy may be manifested by premature ventricular contractions, ventricular tachycardia, or hypotension [1], and bradycardia due to auriculoventricular block [3]. In one patient [1], the electrocardiogram showed ventricular hypertrophy and in another [3], a left bundle branch block. Echocardiogram showed reduced ejection fraction. Intracardiac conduction defects were seen in twin siblings who died after an episode days after onset at two months [12]. Over a period of 25 years, in 107 newborns, cardiac arrhythmia and conduction defects were the main presentations in patients with fatty acid oxidation defects [2]. Conduction disorders and atrial tachycardias were observed in patients with defects of long-chain fatty acid transport across the inner mitochondrial membrane (carnitine palmitoyl transferase type II deficiency [Chapter 38] and CACT deficiency) and in patients with trifunctional protein deficiency (Chapter 41). Arrhythmias have been attributed to the accumulation of intermediary metabolites of fatty acids, such as long-chain acylcarnitines [13].

Muscle disease may be seen very early in this disorder [4]. Weakness and hypotonia may be manifest first in poor head control, and later an inability to walk further than 15 feet [1]. The level of creatine kinase (CK) in the blood may be elevated [4, 10, 14]. Hypertonia may develop during terminal coma [1].

Hepatomegaly is a regular occurrence in this disease, and size tends to increase with time. Hepatic failure

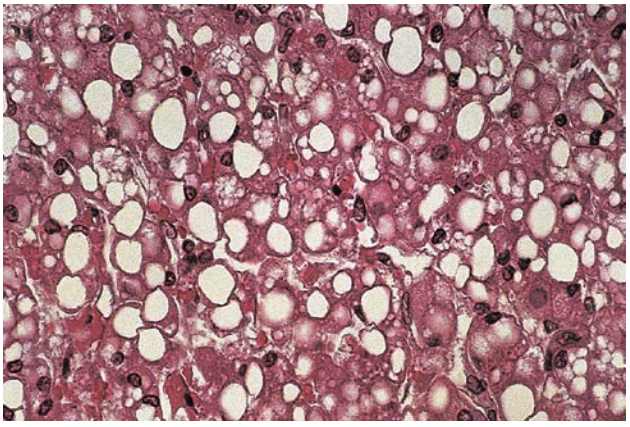

Figure 36.3 Biopsied liver of a patient with CACT deficiency. This H&E-stained section reveals extensive deposition of fat. (This illustration was kindly provided by Dr Jean-Marie Saudubray of Paris.)

has also been recorded [2, 14]. In one patient, there was nephromegaly. Histologic examination of the liver may reveal massive macrovascular steatosis (Figures 36.3 and 36.4) [2, 3], as well as some fibrosis. Muscle histology has been normal. Mental development and growth have been normal [1], but most of these patients have died in infancy. One patient [10] developed microcephaly. Terminal episodes in most were cardiorespiratory failure and cardiac arrhythmia. In one, there was a pulmonary hemorrhage and death at eight days of life [3].

In addition to the hypoglycemia and deficient ketogenesis, clinical laboratory data have included hyperammonemia (491, 270, and 272 μmol/L) [1, 3, 14]. Patients were treated with sodium benzoate [1, 14]. In some patients, a urea cycle defect was considered. Some have had hyperammonemia in infancy without hypoglycemia or any of the other manifestations of a deficit in fatty acid oxidation [14]. Orotic acid excretion was, however, normal [9].

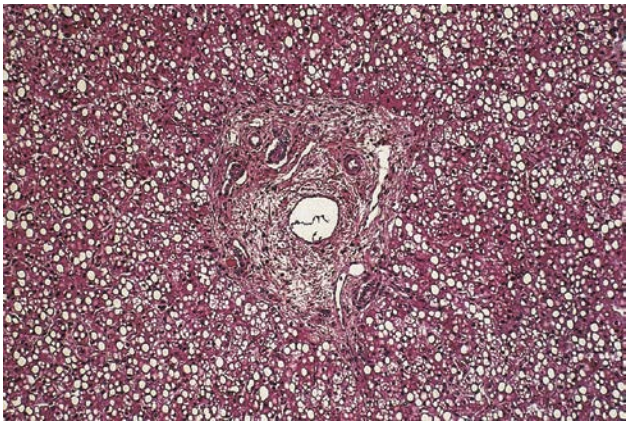

Figure 36.4 Biopsied liver. The fat followed both microvesicular and macrovesicular patterns of steatosis. (This illustration was kindly provided by Dr Jean-Marie Saudubray, Paris.)

During the acute episode, creatine phosphokinase levels in the blood as high as 4595 IU/L have been recorded, and levels remained over 500 between episodes. Acute elevations of uric acid have not been reported, but our experience with other disorders of fatty acid oxidation leads to a prediction that if measured they would be high [15]. Transaminase activities in the blood, both alanine and aspartate amino transferases, have been consistently elevated. Plasma concentrations of free carnitine are low, and the esterified carnitine of blood and urine elevated. In the plasma, the long-chain acyl carnitine fraction was elevated. Urinary organic acid analysis may be unremarkable [1, 3], or there may be mild dicarboxylic aciduria (C6, C8, C10, C12, and unsaturated C10 and C12) [4, 14]. At times of acute episodes, 3-hydroxydicarboxylic aciduria may also be seen [16]. In response to continued fasting, failure of ketogenesis was observed, along with a relative paucity of dicarboxylic acids [1]. Analysis of organic acids after the acute hypoglycemic episode has been successfully treated is usually normal [17].

The advent of acylcarnitine profiles by tandem mass spectrometry has made the diagnosis of this disease considerably easier and more reliable (Table 35.1). The pattern is dominated by the elevation of long-chain acylcarnitines, especially C16 and C18:1 and the deficiency of C0, free carnitine (Figure 36.5) [4]. CPT 2 deficiency has an identical pattern, so enzyme assay is required for definitive diagnosis. Retrospective diagnosis of translocase deficiency has been made by the acylcarnitine profile of a neonatal blood spot (Figure 36.6) [11].

A mild phenotype has been reported [16] in the seventh born of first cousin Pakistani parents. The potential lethality of even this variant is indicated by the fact that the fifth child of this union died at three months, and the sixth had seizures, respiratory distress, and an undetectable glucose at 48 hours, and he died of ventricular tachycardia later that day. Autopsy showed severe steatosis of the myocardium, as well as of the liver and kidneys. The patient reported was diagnosed by tandem mass spectrometry of a neonatal blood spot in which palmitoylcarnitine was 8.85 μmol/L (normal, 4.82) and C2 and C0 were low. A controlled fast at four months revealed elevated free fatty acids without increase in 3-hydroxybutyrate and increased dicarboxylic and 3-hydroxydicarboxylic acids in the urine. Despite frequent feeding and attempted avoidance of fasting, he had a hypoglycemic seizure at 12 months. Cornstarch was added to the night-time regimen at two years, and he was developing normally at three years of age. Another patient with a mild phenotype was reported at five months of age [18].

Assessment of the concentrations of long-chain fatty acylcarnitine esters has become the gold standard for the diagnosis of disorders of fatty acid oxidation [17]. Associated deficiency of carnitine may be severe.

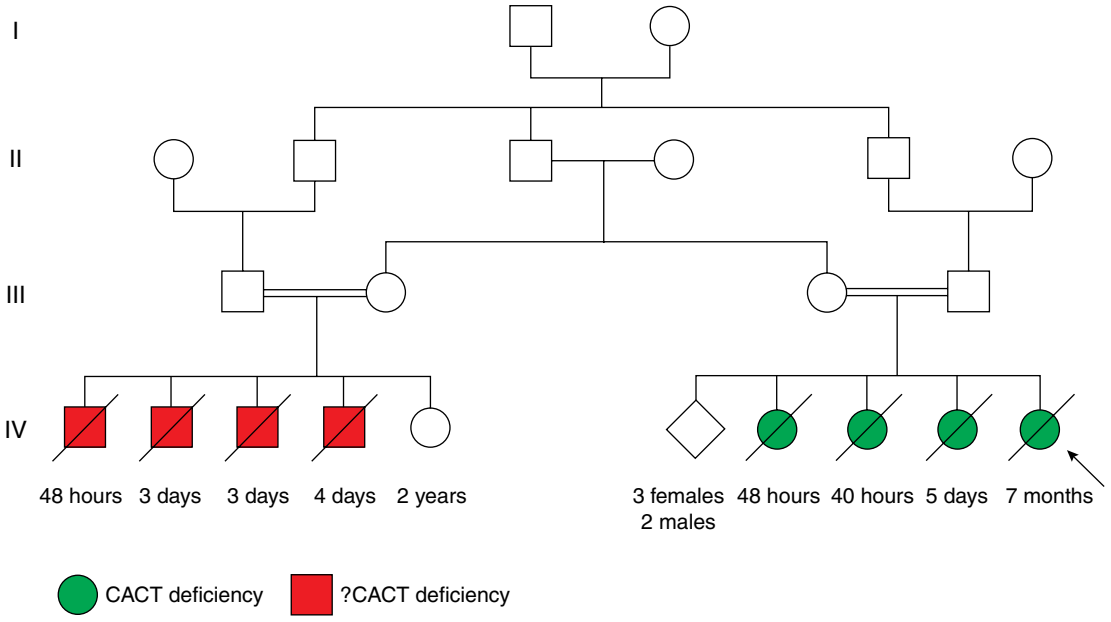

Figure 36.5 Family pedigree of the above patient with CACT deficiency is seen in two branches of the family; all previously affected neonates had died with the disease.

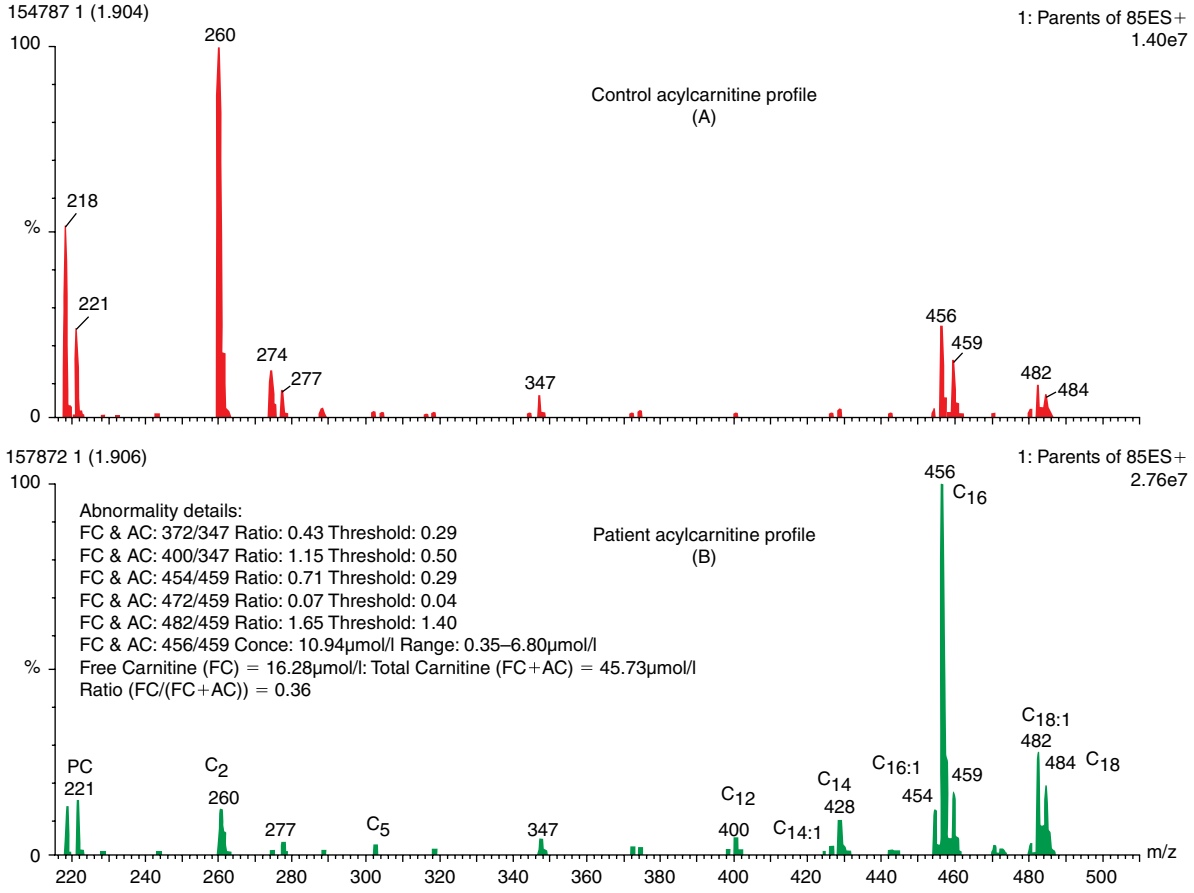

Figure 36.6 Acylcarnitine profiles of blood spot of a patient with CACT deficiency and a control. In the patient carnitine was very low and long chain acylcarnitines high (peaks, 456–484). The peaks in the profile are the molecular ions (M+) of the free carnitine peak 218, and acylcarnitine butylesters (peaks, 260–484). Assessment for the concentrations of long chain fatty acylcarnitine esters has become the gold standard for the diagnosis of disease of fatty acid oxidation [17]. Associated deficiency of carnitine may be severe [17].

GENETICS AND PATHOGENESIS

The fundamental defect in CACT may be demonstrated in cultured fibroblasts [1]. In the first patient, activity was barely detectable at 0.8 percent of the normal mean. Prior incubation with digitonin, to increase permeability, indicated the fibroblast assay to be linear with time and protein and that, with this assay, the activity in the patient studied was zero [3]. In 12 patients, the activity was less than 1 percent of control in all but one [4]. In that patient, the one with the mild phenotype [16], activity ranged from 3 to 6.8 percent of normal. The enzyme can be assayed in fresh lymphocytes and in amniocytes [3, 4].

Intermediate levels of activity were found in fibroblasts of the mother and father of the first patient approximating 50 percent of control, consistent with an autosomal recessive mode of genetic transmission [1], as was consanguinity in other kindreds [3, 16]. However, overlap between control and parent levels has been observed [4]. Better discrimination has been obtained when the results were expressed as the ratio of values of pyruvate conversion to acetylcarnitine or citric acid cycle intermediates [11].

In vitro, the oxidation of fatty acids by fibroblasts or lymphocytes reveals that oxidation of long-chain fatty acids, such as oleate [1] or palmitate [3] were very low, while that of octanoate was normal. Oxidation of palmitate and myristate in the patient with the milder phenotype, while abnormal, was somewhat better [16].

The differentiation of CACT from CPT 2 deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency from mitochondrial trifunctional protein (MTP) deficiency continues to be ambiguous using current acylcarnitine profiling techniques either from plasma or blood spots, or in the intact cell system (fibroblasts/amniocytes). Currently, enzyme assays are required to differentiate CACT from CPT 2, and LCHAD from MTP. Diagnostic elevation of unlabeled butyrylcarnitine may be detected in CACT-deficient cell lines incubated with a shorter chain fatty acid, [7-2H^3] heptanoate plus L-carnitine, rather than routinely used long-chain fatty acid, [16-2H^3] palmitate [19].

The assay developed by Pande *et al.* [3] employs mitochondria purified from intact fibroblasts made permeable with digitonin; in combination with labeled pyruvate ultimate conversion to labeled acetylcarnitine. Another fibroblast-based assay developed by Wanders and colleagues [17] involved the formation of ^{14}C02 from labeled acetylcarnitine.

The pathogenesis of hypoketotic hypoglycemia and cardiomyopathy in patients with fatty acid oxidation (FAO) disorders is still poorly understood. *In vitro* studies are hampered by the lack of natural mutants to assess the effect of FAO inhibition. In addition, only a few inhibitors of FAO are known. Furthermore, most inhibitors of FAO are activating ligands of peroxisome proliferator-activated receptors (PPARs). Aminocarnitine, a carnitine analog, inhibits FAO efficiently, but does not activate PPAR, and it inhibits CPT with different sensitivities towards CPT 1 and CPT 2, as well as CACT [20].

NADPH-cytochrome P450 reductase (CPR) is an essential component for the function of many enzymes, including microsomal cytochrome P450 (P450) monooxygenases and heme oxygenases. In mouse models, a reduced serum cholesterol level and an induction of hepatic P450s were observed, whereas hepatomegaly and fatty liver were only observed in the null model. In addition, induction of a fatty-acid translocase and suppression of CPT appeared responsible for severe hepatic steatosis [21].

Prenatal diagnosis has been reported in six fetuses at risk [4]. Methodology included oxidation of fatty acids and enzyme assay in cultured amniocytes (n = 4) and chorionic villus material (n = 3). One fetus was affected. Results were confirmed in all six. Two other prenatal diagnoses of translocase deficiency have been reported [13].

The translocase enzyme, isolated from rat liver mitochondria, is 32.5 kDa [22]. Its affinity is greatest for C12–6 acylcarnitines [23]. The human translocase cDNA has been cloned [24]. It is 1.2 kb in length and codes for a 301 amino acid (32.9 kDa) protein. The gene is on chromosome 3p21.31 [25]. Mutations in the cDNA sequence have been defined in a small number of patients. A homozygous cytosine insertion causing a frameshift and elongation of the protein by 21 amino acids was found in a patient with a mild phenotype [24]. In a patient with severe disease, there was compound heterozygosity for two extensive deletions [26]. In a patient with severe disease from a consanguineous kindred, in which seven previous siblings had had neonatal deaths, a 558 C to T transition in the cDNA led to a premature stop at amino acid 166 [4]. The transition was confirmed directly in genomic DNA of the patient and her parents [27]. In other severely affected patients, missense mutations have been reported, including G81R and R133W [28,29]. A novel c.609-3c>g (IVS6-3c>g) mutation on the paternal allele was found in compound with a previously described c.326delG mutation on the maternal allele. Most SLC25A20 mutations have been found in a single family [30]. A renumbering of mutations in accordance with recommendations of the Human Genome Variation Society [17] added a homozygous 532C>T transformation (p.R178X), In two Japanese patients with deficiency of the enzyme, one had c.576G>A and c.199-106-2a>t and c.576G>A [31]. In a patient with cardiac arrhythmia, nonketotic hypoglycemia and consanguineous parents, there was homozygosity for a glycine 238 to arginine mutation (Q238R) [32]. In another report [33] patients studied, had five novel mutations and three that had previously been reported. Correlation between phenotype and genotype remained elusive.

Deficiency of carnitine acyl translocase leads to the accumulation of the free fatty acids outside the mitochondrial matrix; long-chain acylcarnitines and short chains are also found, consistent with the fact that purified

translocase catalyzes the transport of short, as well as long-chain acylcarnitines [22]. The long-chain acylcarnitines predominate during illness following fasting-induced lipolysis. Medium- and short-chain esters might reflect the acyl CoA products of peroxisomal oxidation that would require transfer into the mitochondria via the translocase for final oxidation. Secondary deficiency of free carnitine would be expected to result from the excretion over time of large amounts of esterified carnitine.

The hyperammonemia has been associated with normal amounts of orotic acid in the urine. This would suggest an inhibition by accumulated compounds of carbamylphosphate synthetase or acetylglutamate synthetase, as has been shown for propionic acidemia and other organic acidemias [34]. This differs from medium-chain acyl CoA dehydrogenase deficiency in which we have reported hyperammonemia and orotic aciduria [35], suggesting inhibition of the ornithine transcarbamylase step of the urea cycle.

The oxidation of long-chain fatty acids is the chief source of energy during fasting for long periods and for skeletal and cardiac muscle during exercise [36]. The hepatic oxidation of long-chain fats leads to ketone body production, gluconeogenesis, and maintenance of blood levels of glucose during fasting [37]. The clinical manifestations of translocase deficiency are similar to those of the infantile form of CPT 2 deficiency [38] in which a similar acylcarnitine profile is observed.

Dynamic acetylation and deacetylation of nuclear histones is essential for regulating the access of chromosomal DNA to transcriptional machinery. The source of acetylCoA for histone acetylation in mammalian cell nuclei is not clear. Acetylcarnitine formed in mitochondria is transported into cytosol by carnitine/acylcarnitine translocase, and then enters the nucleus, where it is converted to acetylCoA by a nuclear carnitine acetyltransferase and becomes a source of acetyl groups for histone acetylation. Genetic deficiency of the translocase markedly reduced the mitochondrial acetylcarnitine-dependent nuclear histone acetylation, indicating the significance of the carnitine-dependent mitochondrial acetyl group contribution to histone acetylation [39].

TREATMENT

The ultimate courses in many of the patients described have been relentless despite treatment. However, patients with less complete defects have had milder phenotypes. Assessing the outcome of FAO disorders is difficult, as many are rare. For diagnosis by newborn screening, the situation is compounded: far more patients are uncovered by screening than by clinical presentation, representing a somewhat different cohort. Treatment should emphasize the avoidance of fasting and the use of IV glucose to prevent it. Supplemental carnitine and restriction of the intake of long-chain fats are prudent. Early medical intervention in the form of IV 10–25 percent glucose and carnitine supplementation followed by a gradual introduction of a high carbohydrate low fat diet has resulted in good clinical and biochemical response in some patients [8, 9]. Glucose (10%) is infused [40] at rates of 10–12 mg/kg to maintain levels >5 mmol/L [40]. A central line is installed and later a port-a-cath for access in case of an emergency. Insulin plus glucose may be required. Peritoneal or hemodialysis may be required in the acute situation. A permanent Tenckhoff catheter has been employed. The acute hyperammonemia would be expected to respond to sodium benzoate, phenylacetate, or phenylbutyrate. A trial of arginine might be effective. Cornstarch regimens appear to be useful in preventing hypoglycemia. Continuous nocturnal tube feeding is often required [41]. Dietary management emphasizes a high calorie, high carbohydrate intake along with restriction of the intake of fat and substitution of medium-chain triglycerides (MCT) [17]. Docosahexaenoic acid (DHA) may become deficient and require supplementation.

An MCT formula with 18 percent ≥C10 acids and walnut oil has been recommended [41], as opposed to the usual (caprilon) containing, 30 percent [41]). Despite some concerns, carnitine administration has been used without adverse events: levels of carnitine may be very low. Administration of carnitine has been reported [32] to promote normalization of cardiac size and function and prevent further episodes arrhythmia.

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37

Carnitine palmitoyl transferase I deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia; acute episodes leading to convulsions and coma; hepatomegaly; hepatic failure; adult onset myopathy; elevated levels of carnitine and carnitine phosphokinase in blood, and deficiency of carnitine palmitoyl transferase I.

INTRODUCTION

Deficiency of carnitine palmitoyl transferase (CPT) I was first described in 1980 by Bougneres *et al.* [1, 2], in a patient who developed hypoketotic hypoglycemia and morning seizures at eight months of age. They referred to the disorder as deficiency of hepatic carnitine acyl transferase, or palmitoyl transferase, to distinguish it from the deficiency of muscular CPT, in which there is a very different phenotype of muscle pain and rhabdomyolysis, usually observed in adults after exercise [3]. They documented deficient carnitine acyl transferase activity in a biopsied liver. Bonnefont *et al.* [4] clearly distinguished CPT I and CPT II, and demonstrated that CPT I activity was deficient in fibroblasts of the original patient and two others [4–6]. CPT I (Figure 37.1) plays an integral part in the transfer of long-chain fatty acids into the mitochondria, where all the enzymes of β-oxidation are located. The enzyme is situated in the outer membrane of the mitochondrion. In the reaction catalyzed, fatty acyl CoA esters are converted to carnitine esters. Medium- and short-chain fatty acids, in contrast, pass directly into mitochondria and thus do not require esterification with carnitine [7]. CPT II is situated on the inner mitochondrial membrane; it catalyzes the regeneration of carnitine and the long-chain fatty acyl CoAs, which then undergo β-oxidation.

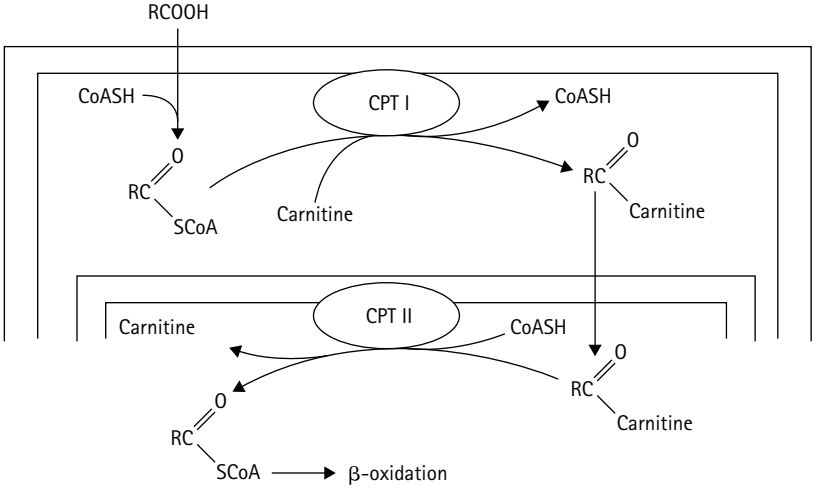

Figure 37.1 The transport of long-chain fatty acids into the mitochondrial sites of β-oxidation involves first the formation of acylcarnitine esters, catalyzed by carnitine palmitoyl transferase (CPT) I; once inside the membranes the liberation of fatty acyl CoA is catalyzed by CPT II. CPT I and II, carnitine palmitoyl transferase I and II; CoASH, coenzyme A; R, fatty acyl side chain.

Carnitine-mediated transport of fatty acids is thought to be rate-limiting in the oxidation of fats. A defect anywhere in the pathway would be expected to lead to inadequate formation of ketone bodies in response to fasting along with inadequate gluconeogenesis and hypoglycemia.

Three isoforms of CPT I have been identified [8]. Type IA or H-I, the hepatic isoform, the key regulator of fatty acid metabolism, is defective in CPT I deficiency. It transports long-chain fatty acyl CoAs across the outer mitochondrial membrane. Affected infants have hypoketotic hypoglycemia, but often remain otherwise well. Newborn screening tests reveal elevated free carnitine (elevated C0/C16 + C18). Confirmation of the leukocyte diagnosis is accomplished by assay of the enzyme in fibroblasts. The disorder is detected by newborn screening, with variable sensitivity [9]. Type IB (M-I) is expressed in skeletal muscle. Fatty acid synthesis in the central nervous system is implicated in the control of food intake and energy expenditure. Malonyl CoA is an intermediate in this pathway. Malonyl CoA is an inhibitor of CPT I. CPT Ic knock out (KO) mice have lower body weight and food intake, and this is consistent with the function of malonyl CoA as an energy sensor. Paradoxically, CPT Ic KO mice fed a high-fat diet become obese and have decreased rates of fatty acid oxidation [10]. CPT Ic is found in the brain. Following a high-fat intake, CPT Ic KO mice developed severe insulin resistance, which was considered a result of increased hepatic gluconeogenesis and decreased uptake of glucose by skeletal muscle. Elevated concentrations of nonesterified fatty acids in plasma are thought to be important mediators [11]. Overexpression of CPT Ic in hypothalamus after injection of a CPT Ic adenoviral vector protects mice from obesity [12], confirming a role for CPT Ic in energy homeostasis.

Carnitine also functions as an acyl group acceptor. This has implications for therapy of disorders of fatty acid oxidation as it promotes export of acylcarnitines from the mitochondria and ultimately urinary excretion. Carnitine requirements increase under metabolic stress. Rodents fed a high-fat diet accumulate acylcarnitine esters and have decreased expression of carnitine biosynthetic genes [13]. This compromises fatty acid β-oxidation. Supplementation with carnitine reversed these abnormalities and improved glucose tolerance.

Human CPT IA cDNA has been cloned [14]. It codes for 773 amino acids in a mass of 88.1 kDa. Mutations have been identified [15, 16].

CLINICAL ABNORMALITIES

This disorder usually presents in infancy, often in the second six months, with acute hypoketotic hypoglycemia during an episode of fasting brought on by an intercurrent, usually viral illness, or gastroenteritis [2, 5, 6, 17, 18]. Onset of symptoms may be neonatal or as late as 18 months. In the first family [2], a previous sister had had three hypoglycemic episodes and had died at 15 months after a 16-hour fast. The hypoglycemic episode may lead to convulsions and coma. Episodes tend to be recurrent until diagnosis and the institution of avoidance of fasting. The disease is potentially lethal. A lethal neonatal presentation has been reported [19].

The liver is usually enlarged, but soft [2]. The acute episode has often been described as Reye-like. Two patients (Figures 37.2 and 37.3) [6] developed predominantly hepatic illness, one (Figure 37.2) at ten months without documented hypoglycemia, in which hepatosplenomegaly and petechiae

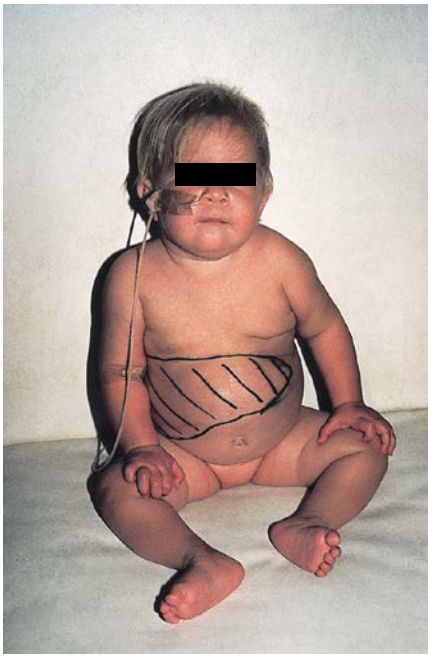

Figure 37.2 SVE: An infant with carnitine palmitoyl transferase (CPT) I deficiency [6]. She had hepatomegaly and hypoketotic hypoglycemia.

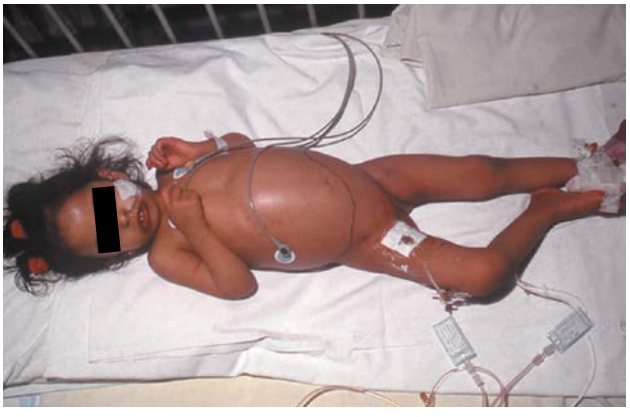

Figure 37.3 A two-year-old Saudi female with carnitine palmitoyl transferase (CPT) I deficiency. She presented with typical Reye syndrome with hepatomegaly, hypoglycemia, and hyperammonemia and very high transaminases, as well as liver failure. She was one of three siblings with the disease. A unique mutation 1950G→A resulted in a glycine 650 aspartic acid change (G650D) in the protein [26]. The family underwent preimplantation genetic diagnosis, and they have had normal children.

were associated with abnormal serum hepatocellular enzymes, prothrombin time, and partial thromboplastin time. She was thought to have disseminated intravascular coagulation and sepsis, consistent with an earlier *Klebsiella* sepsis at two days of age. At 14 months, she developed seizures and was found to have hypoglycemia with no ketonuria.

An interesting hepatic effect of the disease was the occurrence of acute fatty liver of pregnancy (AFLP) in a woman pregnant with each of two siblings found to have CPT I deficiency [20]. This disease then must be added to long-chain hydroxyacylCoA dehydrogenase (LCHAD) deficiency (Chapter 41) as causative of AFLP or the hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. Hyperlipidemia has been reported during acute illness in patients with CPT I deficiency. It may serve as an alerting signal for the diagnosis [21].

Muscle biopsy may reveal lipid storage and vacuoles in electron microscopy [6], but there is no evidence of myopathy and no cardiomyopathy. This disease has been notable for its absence of cardiac symptomatology, including arrhythmias [17]. The neonatal death [19] was attributed to cardiac disease, but the only manifestations of bradycardia and arrest could reflect major systemic illness, and there were no abnormalities in cardiac or skeletal muscle at autopsy.

Renal tubular acidosis has been observed in a number of patients [6, 18, 22]. It may be transient, or associated with myopathy and elevated creatine deficiency [23]. A patient displayed distal renal tubular acidosis, in which there was failure to acidify the urine during spontaneous acidosis [24]. Most patients have survived and there is a tendency to decreased frequency and severity of attacks with time and with learning to avoid fasting. In general, fasting over 15 hours is required to exhaust glycogen stores and call on fatty acid oxidation.

Cognitive deficit, or its absence, generally depends on the severity of the initial hypoglycemic episode, but many patients described have been neurologically impaired [18]. In patients with residual neurologic deficit, the electroencephalograph (EEG) may show focal slowing or spike discharges, and neuroimaging may show cerebral atrophy [6].

MR spectroscopy may show a high N-acetylaspartate creatine ratio [25]. There may be a continuing seizure disorder [22]. Linear growth and anthropometric development tend to be normal.

The creatine phosphokinase (CK) in the blood may be elevated during acute episodes [26], and this has been attributed to the MM isozyme, but the alanine transaminase (ALT) and aspartate transaminase (AST) were even more elevated; the levels of these hepatic enzymes were also elevated in other patients during acute hypoglycemic episodes [6, 23]. Blood sugar levels during the acute attack have been reported at 0.3, 0.9, 1.2, 1.3, and 1.6 mmol/L [2, 5, 6, 22, 24]. In one patient, there were recurrent Reye-like episodes without hypoglycemia, although there was improvement in lethargy on the administration of glucose [22].

Organic acid analysis of the urine is notable for the absence of dicarboxylic aciduria and hydroxydicarboxylic aciduria [2, 6, 18], as well as the absence of elevation of 3-hydroxybutyric and acetoacetic acids in the urine at times of fasting and hypoglycemia. Plasma levels of carnitine may be normal or elevated [25]. Levels of both free and total carnitine may be elevated. Fractionation of the esterified carnitine of the urine reveals only acetylcarnitine. Tandem mass spectrometry of the blood reveals an absence of long-chain acyl carnitines (C16, C18, C18:1) [18, 27]. The ratio of C0 to C16 + 118 is useful in diagnosis [28]. In three patients, the ratio ranged from 175 to 2000, a range of 2–32 was observed in control infants. Higher values were found in older infants, but there was no overlap of patients and controls.

The histologic examination of the liver has revealed microvesicular and macrovesicular steatosis [6, 26]. Muscle biopsy may show sarcolemmal and interfibrillar accumulation of glycogen, as well as the presence of lipid [6]. A hematophagocytic syndrome has been observed in a patient with otherwise clinically typical CPT I deficiency, who developed a brain abscess with candida [29]. This hemophagocytic syndrome has also been observed in propionic acidemia and lysinuric protein intolerance.

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion. Affected children of both sexes have been observed with normal parents. Consanguinity has been documented [2, 26]. In one extended inbred Hutterite kindred, a brother and sister and their second cousin were affected [24]. The disorder is rare [18], but there were nine patients in the Paris experience of Saudubray *et al.* [17] of 107 patients with abnormalities of fatty acid oxidation.

The enzymatic defect in CPT I is most carefully documented by measuring the production of labeled palmitoylcarnitine from methyl-labeled carnitine and palmitoyl CoA in fibroblast homogenates, in which the integrity of mitochondrial membranes is preserved [5, 26]. Testing with and without malonyl CoA distinguishes CPT I, which is inhibited by malonyl CoA, from CPT II, which is not. Reported activity has ranged from 9 to 23 percent of control. CPT I appears to determine the overall rate of oxidation of fatty acids in the liver. Some patients have been documented to have deficiency of CPT I in liver, while activity in muscle was normal [30]. Enzyme assay for confirmation of the diagnosis of CPT I deficiency has been facilitated by the addition of cyanide to the reaction mixture to inhibit the activity of enzymes downstream of CPT I which otherwise degrade the palmitoylcarnitine formed in the assay [31]. Quantification is by tandem mass spectrometry.

In earlier assays, fibroblasts from patients with CPT I deficiency incubated with labeled palmitate accumulated labeled palmitoyl CoA, but not palmitoyl carnitine [32]. The fibroblasts of CPT I-deficient patients display defective overall oxidation of long-chain fatty acids, such as palmitate, whereas oxidation of octanoate and succinate was normal [5, 33]. Similarly, the conversion of ^{3}H-palmitate to $^{3}H_2O$ in fibroblast monolayers was markedly deficient [33]. These observations are consistent with the failure of CPT I-deficient cells to transport long-chain fatty acids into mitochondria, while medium-chain compounds are transported normally.

Immunochemical studies have been carried out with antibodies against CPT I and CPT II [34, 35]. CPT I has been demonstrated immunochemically in rat liver and kidney. Immunochemically, CPT I is absent in muscle and heart, which provided early evidence for the presence of the muscle-specific isoform. The hepatic isoform approximates 88 kDa in size, while that of muscle is approximately 82 kDa; both variants are found in heart.

Parents of affected siblings have been found to be intermediate in levels of CPT I activity in fibroblasts, consistent with heterozygosity. Prenatal diagnosis has not been reported, but preimplantation genetic diagnosis has yielded normal offspring (Figure 37.3).

The fatty acid transport protein FAT/CD36 found in the plasma membrane may also contribute to the regulation of fatty acid oxidation. FAT/CD36 KO mice are unaffected by sulfo-N-succinimidyloleate which inhibits palmitate transport across the plasma membrane [36]. Regulation of mitochondrial fatty acid oxidation is particularly relevant during the metabolic demands of muscle contraction. The CPT IA gene on chromosome 11q13.1-13.5 [37] is expressed in liver, kidney, pancreas, ovary, leukocytes, and fibroblasts [16]. The gene spans 60 kb and contains 20 exons. The first mutation described [15] was a missense (D454G) change, which when expressed, had 2 percent of wild-type activity. Other mutations identified [16] include Q100X, which would predict an early truncation of the protein, H414V, and Y498C, which affect highly conserved sequences in the catalytic core of the enzyme. An 8-kb deletion encompassing intron 14 to exon 17 led to loss of the mRNA [16]. The rarity of the disease and the general severity of phenotype have made genotype–phenotype correlations difficult, but the mutation leading to P479L resulted in a late onset disease in which there was proximal myopathy. Homozygosity for the 1436 (C > T) mutation was identified [38] in patients with deficient CPT 1A enzyme. In a Japanese newborn, two novel mutations, p.R446X and p.G719D were found [39]. Four novel mutations were found [40] in patients with severe disease: G405W, R316R, and F343V.

Greenberg and colleagues [41] have addressed the high frequency of the P479L variant in Canada among the Inuit and First Nation families [42]. In the Greenland Inuit, the frequency of this allele was 0.73 in contrast to its complete absence in the nonaboriginal population. Clinical manifestations have tended to be minor or absent. Studies of fatty acid oxidation in fibroblasts revealed residual activity of the enzyme which should be sufficient to permit flux through the mitochondrial oxidation system.

The p.P479L was associated with elevated high-density lipoprotein (HDL) and apoA-1 levels in plasma [42]. It has been suggested that the polymorphism might protect against atherosclerosis.

TREATMENT

The major element in management is the studied avoidance of fasting. In the presence of intercurrent infection or other cause of vomiting or anorexia in which the oral route is excluded, the provision of intravenous (IV) glucose is essential. Reduction of the intake of long-chain fats appears prudent. Medium-chain triglycerides (MCT) may be substituted (Figure 37.4). Admistration of MCT during acute symptomatic hypoglycemia restored normoglycemia and led to brisk ketosis [5]. The ideal concentration of glucose is at least 10 percent, and 25 percent has been recommended [43], along with IV carnitine (Figure 37.5). With recovery from the acute episode, a high-carbohydrate low-fat diet supplemented with MCT is introduced [28]. Acute hyperammonemia may be managed by infusion of L-arginine. Cornstarch regimens are useful in preventing hypoglycemia.

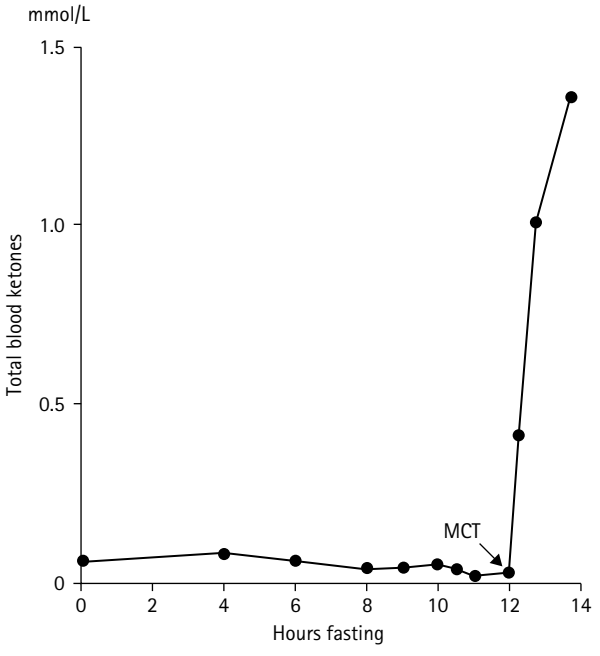

Figure 37.4 Absence of ketogenesis with fasting to hypoglycemia in carnitine palmitoyl transferase (CPT) I deficiency and the brisk ketogenic response to medium-chain triglyceride (MCT). The blood sugar also rose after MCT.

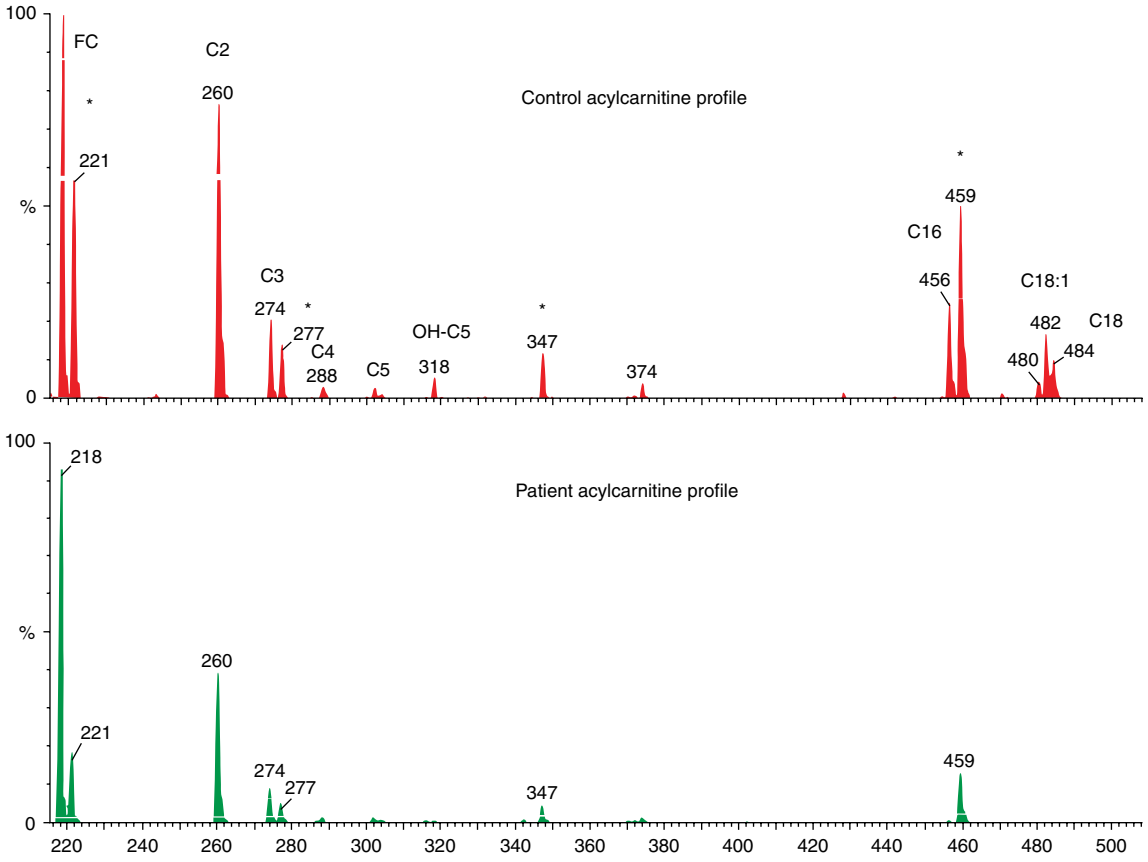

Figure 37.5 Acylcarnitine profiles assayed in blood spots of patients with carnitine palmitoyltransferase (CPT) I deficiency. The very high free carnitine and almost absent long chain acylcarnitines (peaks 456–484) are illustrated. The peaks in the profile are the molecular ions (M+) of the free carnitine peak 218 and acylcarnitine butyl esters (peaks 260–484).

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38

Carnitine palmitoyl transferase II deficiency, lethal neonatal

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, enlarged polycystic kidneys, hepatomegaly, microcephaly, prominent forehead, over folded helices, cardiomegaly, cardiac arrhythmias, elevated long-chain acylcarnitine esters, and profoundly decreased activity of carnitine palmitoyl transferase II (CPT II).

INTRODUCTION

This distinct phenotype of CPT II deficiency, first described by Hug and colleagues in 1989 [1, 2] provides another example of the effects of an inherited disorder of metabolism on early intrauterine organogenesis. In addition to hypoketotic hypoglycemia and hepatomegaly, affected infants have cardiomegaly and cardiac arrhythmias. They also have dysmorphic features and massively enlarged cystic kidneys [1–3]. The brain is also dysplastic and cystic [4–7]. Death in infancy has been uniform, with some exceptions [8].

The activity of CPT II (see Figure 38.1) is markedly reduced in many tissues, including cultured fibroblasts [4–7]. This leads to a characteristic profile of acylcarnitines in which there is elevation of all long-chain species, particularly C16, C18:2, C18:1, and C18 (Figure 38.1). This feature permits the detection of the disease by expanded programs of newborn screening by tandem mass spectrometry (MS/MS) [9].

The gene is located on chromosome 1p32. At least 25 mutations have been detected [10], including 11-bp duplication [11], a 2-bp deletion [6], and compound heterozygosity for two truncating mutations [12].

CLINICAL ABNORMALITIES

The disease typically presents in the first days of life with lethargy, hypotonia, or seizures, and the infant is found to have hypoglycemia, hyperammonia, or both [1–4]. The concentration of ammonia may exceed 1000 μmol/L [4]. Hypoglycemia is classically hypoketotic, and the urine test for ketones is usually negative [4]. Neonatal onset patients have generally died by one month of age (Figures 38.2–38.7). A few patients have had a more classic disorder of fatty acid oxidation phenotype with onset of hypoketotic hypoglycemia at five to ten months following an intercurrent illness that led to prolonged fasting [12–14]. Nevertheless, most of these patients or their siblings have died in infancy. There have been two notable exceptions [12–14].

The massively enlarged polycystic kidneys are a major manifestation of neonatal onset patients (Figure 38.2) [3, 4]. Pregnancy may be complicated by oligohydramnios [4]. The kidneys may be visible, readily palpable, and shown to be polycystic by ultrasound [4]. Prenatal diagnosis has been accomplished by fetal ultrasonography [13–16]. Hyperkalemia may signal rapidly progressive renal failure. One patient [17] had neonatal hypothermia, hyperkalemia, cranial dysmorphism, with heart block.

Hepatomegaly is also characteristic [1, 2, 4], and liver size may increase progressively. Aminotransferase levels may be elevated. Hepatic calcifications have been seen on ultrasound [12]. Histologic examination has revealed lipid vacuoles in hepatocytes [4, 6].

Cardiomegaly may be associated with arrhythmias [1–3, 6] and cardiac failure. Lipid accumulation has been documented in cardiac myocytes [4, 6]. Left ventricular wall and septum were hypertrophied. Skeletal muscle

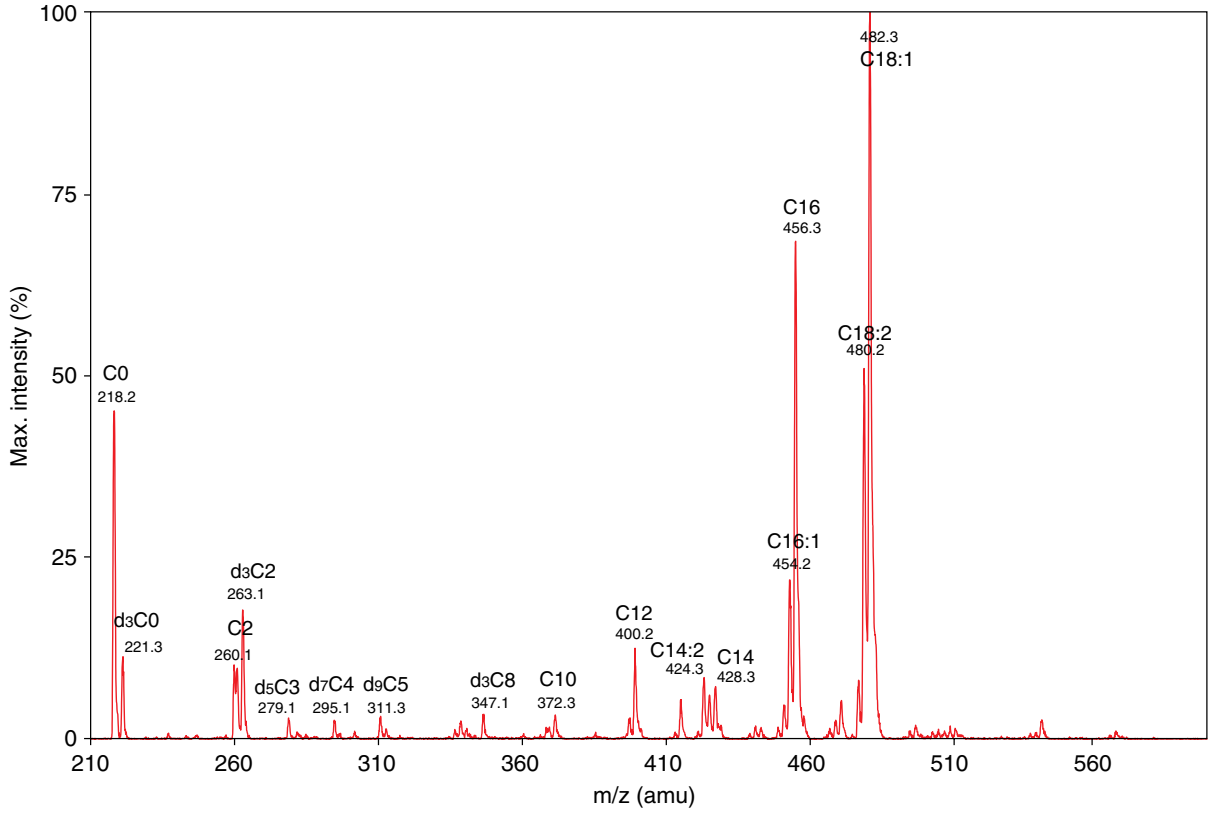

Figure 38.1 Acylcarnitine profile of a neonate with lethal carnitine palmitoyl transferase (CPT) II deficiency.

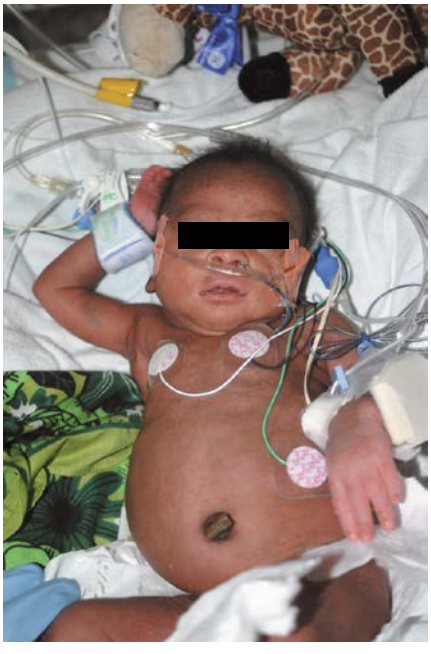

Figure 38.2 Baby T: A neonate with carnitine palmitoyl transferase (CPT) II deficiency who died a few days later at 12 days of age. The enormous kidneys visible in the abdomen were recognized as polycystic on prenatal ultrasound. The liver was also palpable 2 cm below the costal margin. She had hypotonia and microcephaly. She also had cardiomyopathy and pronounced cerebral ventriculomegaly. This infant, also shown in Figures 38.3–38.7 was Case 2 in Isackson *et al.* [13].

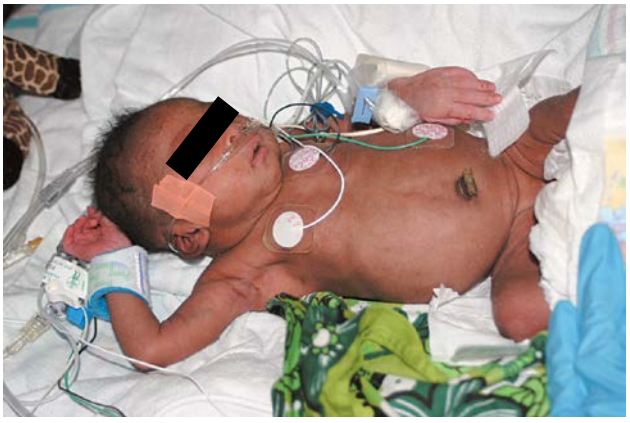

Figure 38.3 Baby T: The ears were low set and rotated posteriorly. The forehead appeared long and sloped backwards.

also showed depositions of lipid [4, 6]. A patient with a myopathic presentation who died at 34 days had deficiency of CPT in muscle [18]. Creatine kinase activity may be increased [16].

Dysmorphic features have included microcephaly, a high sloping forehead, flat occiput, low set ears with or without over folded helices, bulbous nose, long tapering fingers and toes, extra digital creases on fingers 2 to 4 bilaterally, widely spaced nipples, hypoplastic toenails, and contractures of the knees, elbows, and small joints of the hands [4]. Another infant [16] had a high arched narrow palate.

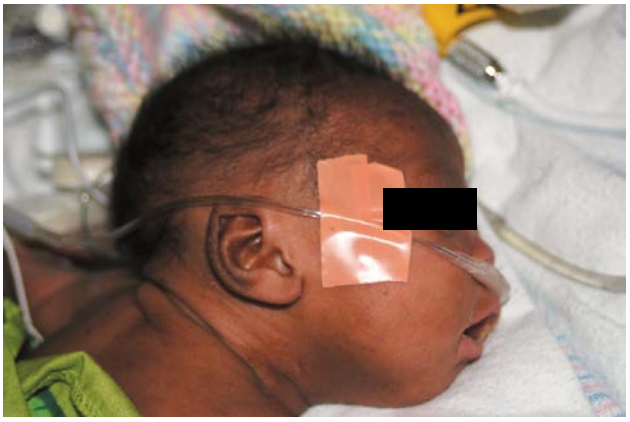

Figure 38.4 Baby T: The helix came to a point just under the nasal catheter.

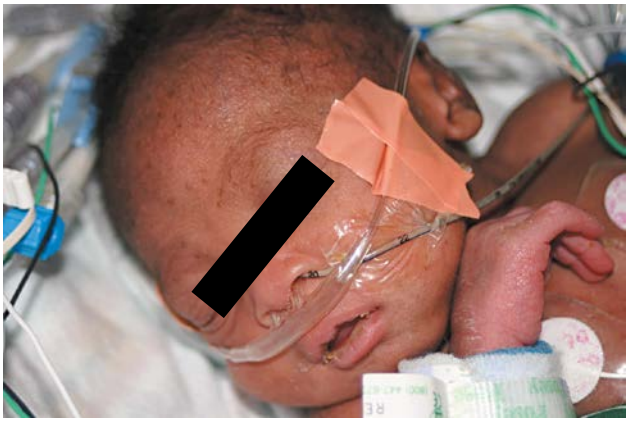

Figure 38.5 Baby T: The nose appeared bulbous.

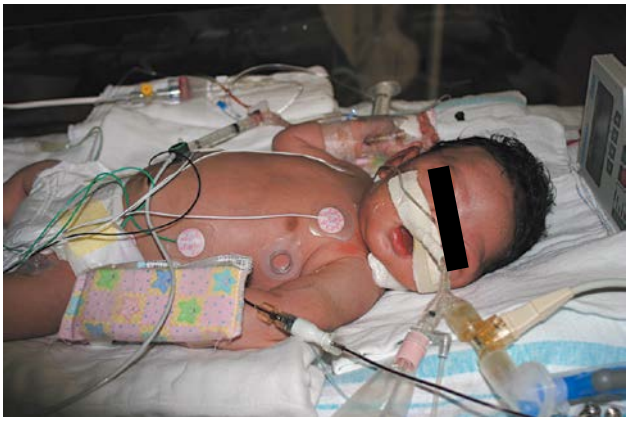

Figure 38.6 BR: A neonate with carnitine palmitoyl transferase (CPT) II deficiency who died at 14 days of age. She had a high backward sloping forehead and a bulbous nose. Renal failure is evident in facial edema. Nipples were widely spaced. Polycystic kidneys were evident on ultrasonography: but this was subtle and the kidneys were not palpable, even during hyperammonemic coma.

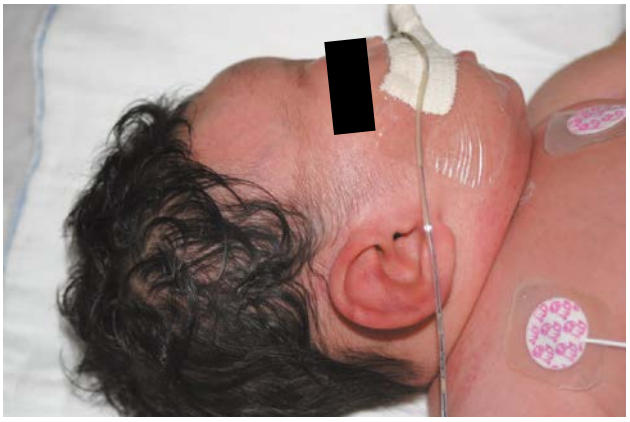

Figure 38.7 The ear was not low, but it was posteriorly rotated. The patient presented on the first day of life with hypoglycemia and hyperammonemia, which responded promptly to the infusion of arginine.

Cystic dysplasia of the brain has also been reported [4, 7], as has polymicrogyria and intracerebral hemorrhage [6]. Cysts may be visible on cranial ultrasonography [4, 7]. On electron microscopy, paraventricular cysts were lined with dense gliosis. There were glial heterotopias at the base of the brain [4]. Neuronal migration abnormalities have been reported [4, 7, 19]. Pathologic findings have been said [10] to be similar to that of patients with severe deficiency of electron flavoproteinubiquitone oxidoreductase (ETF.Q0).

GENETICS AND PATHOGENESIS

CPT II activity has been documented to be decreased in cultured fibroblasts and tissue homogenates. Enzyme activity is generally measured in the forward and reverse directions [20–22], and CPT I activity has been demonstrated by its malonyl CoA sensitivity in the forward reaction. Profound deficiency of CPT II activity found in this phenotype [14, 23] contrasts with the partial deficiency that characterizes the CPT II deficiency that presents with intermittent rhabdomyolysis, in adolescence or adulthood (Chapter 40) [24].

Long-chain fatty acids require a carnitine transport system in order to gain entrance to the mitochondrial matrix where β-oxidation takes place. CPT II is located on the inner side of the inner mitochondrial membrane. It catalyzes the conversion of long-chain acylcarnitine esters, like palmitoylcarnitine, to free carnitine and the corresponding CoA ester, such as palmitoyl CoA.

Concentrations of acylcarnitines are elevated in blood and tissues [4, 7, 12]. In addition to the elevations of especially C16 to C18 (Figure 38.1), the ratios of C16/C8:1 and C18:1/C8:1 are enormously elevated. This pattern may also be seen in carnitine-acylcarnitine translocase (CACT) deficiency (Chapter 36).

Urinary organic acids are usually normal. A few patients have had medium-chain dicarboxylic aciduria,

but without hexanoylglycine or suberylglycine. Plasma and urinary total carnitine may be elevated, particularly the esterified fraction. Free carnitine in blood and tissues is usually normal, or even significantly elevated [13], but it may decrease rapidly [4].

Oxidation of palmitic acid and myristic acid in cultured fibroblasts is decreased [12, 14]. Molecular genetic analysis has been characterized by major disruption of the gene. An 11-bp duplication was found [11] in two siblings [15]. Two truncating mutations were found in an Ashkenazi Jewish infant who died on the third day of life [12]. A number of other mutations found in Ashkenazi infants included the 2-bp deletion and a missense mutation in exon 4 [16]. Compound heterozygosity for these mutations has been found in Ashkenazi patients with the adult form of CPT II deficiency [25]. Two truncating mutations were found in an Ashkenazi Jewish infant [13]. Prenatal diagnosis has been accomplished using fetal ultrasound visualization [26, 27] and by chorionic villus sampling [28].

TREATMENT

The neonatal presentation has led uniformly to early death. A potential exception is a patient who was living at the time of report at 14 months following treatment of acute decompensation with exchange transfusion, along with a long-term high calorie diet supplemented with medium-chain triglyceride [29]. This patient had a unique mutation, a 24-bp deletion leading to deletion of amino acids 179–186 and substitution of phenylalanine for leucine at 178. A major goal of treatment is to supply enough glucose to prevent lipolysis [30]. Glucose has been provided by intravenous or nasogastric administration to maintain high levels of glucose. Glucose plus insulin and repeated exchange transfusions have been employed with reported success [8]. Carnitine treatment seems rational.

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39

Medium-chain acyl CoA dehydrogenase deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, cardiomyopathy cardiac arrhythmia, sudden infant death syndrome (SIDS), myopathy, hyperammonemia, hyperuricemia, elevated creatine kinase, dicarboxylic aciduria, elevated C6 and C8acylcarnitine, deficient activity of medium-chain acylCoA dehydrogenase, and mutation in the *ACADM* gene, especially *A985G*.

INTRODUCTION

Medium-chain acylCoA dehydrogenase (MCAD) (Figure 39.1) (Table 39.1) deficiency is the classic disorder of fatty acid oxidation, and it is the most common. Occurring in an estimated 1 in 6000 to 10,000 Caucasian births, the disease was nevertheless first described in 1983, [1, 2] an index of the difficulties, even today, in detecting disorders of fatty acid oxidation [3].

Disorders of fatty oxidation display two general types of presentation. The first, hypoketotic hypoglycemia, is the clinical picture of Reye syndrome. In fact, it is now clear that most patients who appear to have Reye syndrome have an inborn error of metabolism, the most common being MCAD deficiency and ornithine transcarbamylase deficiency (Chapter 26) [4, 5]. The other presentation reflects the chronic disruption of muscle function with symptoms relevant to myopathy or cardiomyopathy, including weakness, hypotonia, congestive heart failure, or arrhythmia. Both types of presentations may be seen in the same family or even in the same individual. Another presentation is with SIDS [6–9]. We and others have been able to make retrospective diagnoses of MCAD in infants who had died of SIDS by retrieval of neonatal screening blood spots after making the diagnosis of MCAD deficiency in a subsequent sibling and assay for the common mutation in the DNA or for octanoylcarnitine. The introduction of the tandem mass spectrometric analysis of acylcarnitines has greatly facilitated the diagnosis of this and other disorders of fatty acid oxidation, and its application to the screening

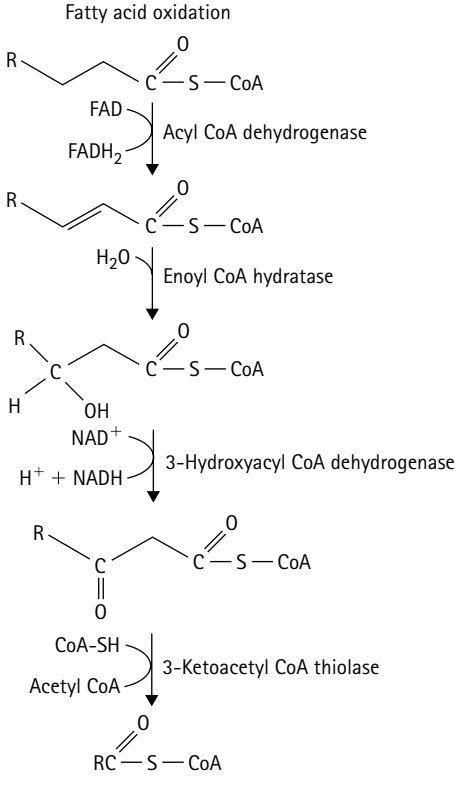

Figure 39.1 The pathway of β-oxidation of fatty acids begins with the acyl CoA dehydrogenase step.

Table 39.1 The acyl CoA dehydrogenases and their substrate specificities

Fatty Acid Oxidation Acyl CoA Dehydrogenases		
Enzyme	**Substrate chain length**	**Deficiency disease**
Short Chain (SCAD)	C4-6	Rarely symptomatic
Medium Chain (MCAD)	C6-12	Common (1: 10,000)
Very Long Chain (VLCAD)	C14-20	Uncommon

of newborns is a major addition to preventive medicine. This could prevent many further examples of SIDS due to MCAD deficiency.

The gene was cloned in 1986 [10] and assigned to chromosome 1. A majority of caucasian patients have the A985 gene mutation.

CLINICAL ABNORMALITIES

Episodic illness usually occurs first between six months and two years, usually following fasting for 12 hours or more as a consequence of intercurrent infectious disease (Figures 39.2–39.4). The episode may be ushered in with vomiting or lethargy, or it may begin with a seizure. It is progressive rapidly to coma [11]. Patients are typically hypoglycemic. Hypoglycemia with a simultaneous negative urine test for ketones is very helpful in diagnosis.

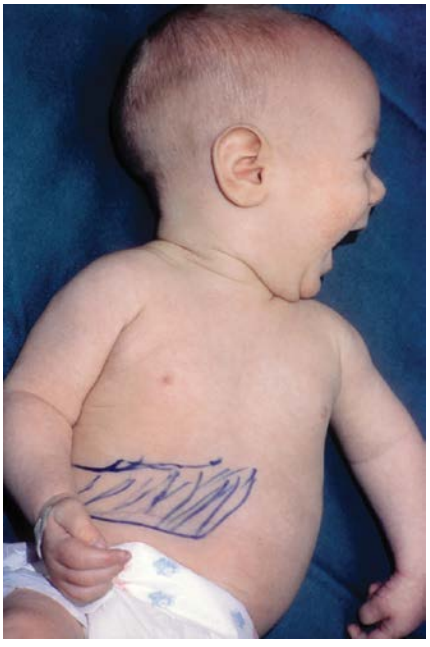

Figure 39.2 A five-week old boy with MCAD deficiency who had presented a few hours earlier with a hypoglycemic seizure; with treatment he was rescued early. He had a big smile and a big liver, which then resolved over a few days.

Figure 39.3 SE at 26 months. Over the previous 18 months, she had suffered multiple episodes of hypoglycemia , however, these were behind her; she was still receiving treatment with carnitine and cornstarch.

However, although these patients are documentable hypoketotic (Figure 39.5), the urine often contains some ketones at times of acute illness; so, this can be misleading [12]. Hepatomegaly is usually present at the time of the acute illness. Liver biopsy at the time reveals abundant

Figure 39.4 KB: A one-year old patient with MCAD deficiency. She presented at seven months with a life-threatening episode of illness. Her sibling died in the first days of life of SIDS and was documented retrospectively to have MCAD deficiency. Once diagnosed, this patient had not had another episode requiring admission to hospital. Nevertheless, the dangerous nature of this disease is signified by the fact that she died at home during sleep without evident prior illness.

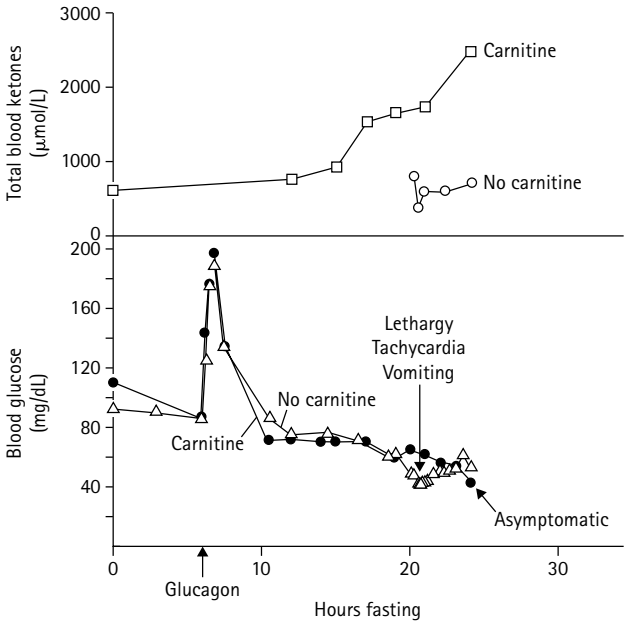

Figure 39.5 Fasting ketogenesis in a patient with MCAD deficiency. A second fast was initiated after initiation of treatment with carnitine, and although she again became hypoglycemic, she had a better ketogenic response, did not have hypoglycemia until 24 hours, and did not develop clinical evidence of illness.

deposits of lipid in microvesicular pattern [13]. This and hyperammonemia have often led to a diagnosis of Reye syndrome [2, 4, 14–16]. Cerebral edema and herniation have been reported in an acute lethal episode [15]. In at least one patient [14], a documented accompanying orotic aciduria permitted fulfillment of published criteria for a diagnosis of ornithine transcarbamylase deficiency, but assay of this enzyme in biopsied liver revealed normal activity. This Reye-like presentation is often the first manifestation of disease in this disorder [17].

During the intervals between episodes of illness, patients typically appear completely well. There is no muscle weakness. However, some patients are impressively hypotonic and display a reluctance to exercise or poor muscle strength. Clinical myopathy or cardiomyopathy is unusual in this condition, particularly early [17], but these problems may develop in any patient with a disorder of fatty acid oxidation. Acute cardiac arrhythmia may be seen at the time of episodic illness.

The issue of arrhythmia in MCAD deficiency has received less than optimal attention in the literature, possibly because so many patients diagnosed early do so well. Rice and colleagues [18] reported a three-day old with ventricular tachyarrhythmia and torsades de pointes refractory to medication that ultimately responded to extracorporeal life support and intravenous (IV) carnitine. We have seen arrhythmia visualized on monitoring in the intensive care unit during an initial hypoglycemic episode. We have also wondered about all those infants with SIDS, like the ones who have been documented on retrieved blood spots once a subsequent sibling has been diagnosed. Arrhythmia appears to be a more logical cause of SIDS than hypoketotic hypoglycemia.

Iafolla and colleagues [19, 20] have assembled data on 120 patients with MCAD deficiency referred for diagnostic testing. The mean age of onset was 12 months, and the range was two days to 6.5 years. Of 120 patients 23 died before the diagnosis was made; 12 siblings of patients had died previously, 11 were diagnosed as SIDS and one as Reye syndrome. Emergency care or admission to hospital was required at onset in 95 percent of patients. Initial symptoms were lethargy in 84 percent, vomiting in 66 percent, and encephalopathy in 49 percent and respiratory arrest in 48 percent. Cardiac arrest was the initial presentation in 36 percent and sudden death in 18 percent. Seizures were present in 43 percent and hepatomegaly in 44 percent.

The acute severity of presentation contrasts with the fact that there were no deaths in 97 surviving patients for an average of 2.6 years following diagnosis. Most of our patients have not had a second episode requiring admission to hospital. On the other hand, in follow-up data on 73 patients older than two years of age [19], there was appreciable long-term morbidity. Twenty-nine percent were judged developmentally delayed; of these, 12 had global developmental disability. Another seven had behavioral problems. Seizure disorder was present in 14 percent and attention deficit disorder in 11 percent.

It has become increasingly evident that a somewhat different clinical picture is emerging of adults, born before the advent of newborn screening, who had escaped an early infantile presentation [21]. In a review of 14 such patients [21], mortality was 50 percent in those presenting acutely and 29 percent overall. Precipitating factors included alcohol, as well as fasting. Cardiac arrhythmias and cardiac arrest were prominent features, as was rhabdomyolysis. These latter features, more prominent in defects of carnitine transport and long-chain fatty acid oxidation, could be related to long-term depletion of carnitine. In two patients, cardiac symptoms resolved after supplementation with carnitine.

Key elements of the clinical chemistry in suggesting a disorder of fatty acid oxidation, are markedly elevated levels of uric acid and creatine kinase (CK) [11, 21–24]. These values are elevated only during the acute episodes of illness when they are very high. Uric acid concentrations are often over 10 mg/dl and have been as high as 20 mg/dl. CK may be over 1000 U/l. These data, along with evidence of large amounts of urate in the urine, indicate that the mechanism is cellular breakdown. Hyperuricemia and high levels of CK have been observed in patients with rabies [25]. In patients suspected of having a disorder of fatty acid oxidation, it is important to order these two alerting tests specifically, as they are usually omitted from panels of clinical chemical tests in children's hospitals.

Carnitine deficiency is the rule in this disease and may be helpful in suggesting the diagnosis. Concentrations of free carnitine are very low in plasma. Levels are also low

in tissues, but muscle biopsy is not commonly available in this disorder. Levels of esterified carnitine are very high in the urine, and this is the mechanism of the secondary carnitine deficiency. In any condition in which the CoA esters of carboxylic acids accumulate, esterification with carnitine takes place, and carnitine esters are preferentially excreted in the urine. This serves a detoxification function, but it also depletes supplies of carnitine, leading to a second mechanism of impaired fatty acid oxidation. Ratios of ester to free carnitine tend to be high in blood as well as urine, but we find this less useful than the actual levels of free carnitine in the plasma and esterified carnitine in the urine.

Medium-chain dicarboxylic aciduria is the hallmark of the organic acid profile in the urine of the patient with this disease, and it may be diagnostic at the time of the acute episode [15]. The typical pattern is that of large amounts of the dicarboxylic acids, adipic (C6), suberic (C8), and sebacic (C10), as well as the glycine conjugates of hexanoic acid and suberic acid. The very large elevation of suberylglycine may be diagnostic [26, 27]. Dicarboxylic acids as long as C12 (dodecanedoic) acid maybe elevated, and omega-1 oxidation yields the hydroxy acids, 5-hydroxy-hexanoic and 7-hydroxy-octanoic acids [28]. Normal infants and children excrete large amounts of dicarboxylic acids with fasting, but the attendant ketosis is mirrored in very large excretions of 3-hydroxybutyric acid and acetoacetic acid. In contrast, in patients with disorders of fatty acid oxidation, the ratio of dicarboxylic acids to the sum of these two compounds is greater than 1 [2]. Unfortunately, these diagnostic features disappear from the urine with the disappearance of the acute episode, so that by the time the patient is referred for study, the organic acid analysis of the urine is usually completely normal.

Fasting under controlled conditions will reproduce the typical pattern of dicarboxylic acids in the urine. We have developed a protocol for the systematic investigation of patients suspected of having disorders of fatty acid oxidation (Chapter 34). Now, it is never necessary in MCAD deficiency because of the number of simple, less invasive tests that are available. Figure 39.5 illustrates its use in a patient diagnosed prior to the development of these tests. At the time that she developed hypoglycemia after 20 hours, she excreted diagnostic quantities of suberylglycine. The flat curve for the blood levels of acetoacetic and 3-hydroxybutyric acids illustrates the impaired ketogenesis along with the development of hypoglycemia and clinical evidence of illness.

The diagnosis of MCAD deficiency in the absence of illness or fasting has been facilitated by the development of a sensitive method of gas chromatography-mass spectrometry (GCMS) using a stable isotope dilution that permits the measurement of the glycine conjugates hexanoylglycine, suberylglycine, and phenylpropionylglycine even in the normal individuals [27]. The amounts found in patients with MCAD deficiency during remission are often large enough to be diagnostic. Phenylpropionylglycine excretion depends on the conjugation of a product of intestinal microbial metabolism, and we have found that it is usually absent during the acute episode, when most patients are receiving antibiotic therapy. Also, patients not colonized by the anaerobic clostridia that produce phenylpropionic acid will not be distinguishable in this way [29].

The modern approach to the diagnosis that may be used in remission as well as during illness is to examine the blood for specific carnitine esters by tandem mass spectrometry (MS/MS) [28, 30–32]. The test is usually carried out on blood spots in programs of newborn screening. For definitive diagnosis, the analysis of plasma is preferable. Octanoylcarnitine is the compound on which reliance is usually placed (Figure 39.6) (see Table 34.1), Hexanoylcarnitine is also useful, and the ratio of C8 to C8:1 is often the best discriminator [30, 33, 34]. Normal newborns have levels under 0.22 μmol/l of octanoylcarnitine (C8). The mean of 16 patients with MCAD deficiency was 8.4 (range 3.1–28.3) μmol/l [30]; and of 35 patients 3.0 (0.4–21.8) μmol/l [33]. A level over 0.3 has been considered a diagnostic criterion for MCAD deficiency. With time and depletion of carnitine, levels of C8 carnitine decrease in patients with MCAD deficiency.

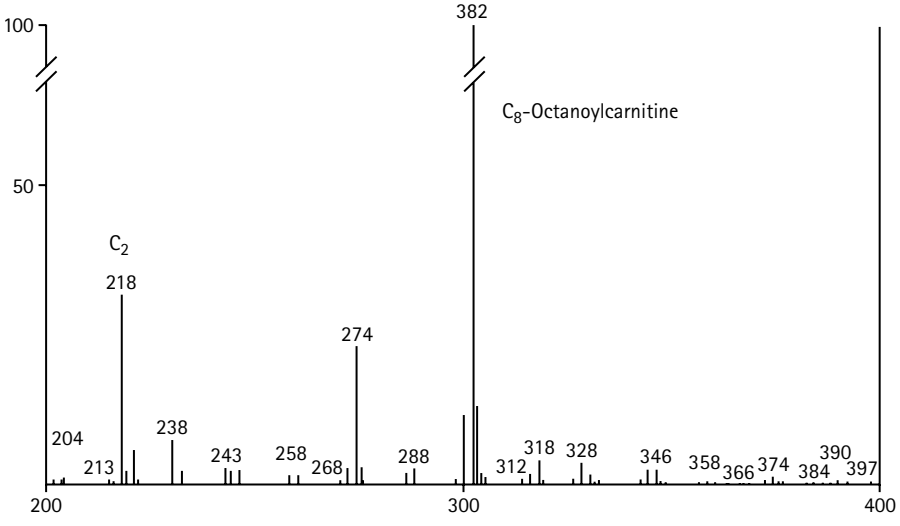

Figure 39.6 Acylcarnitine profile of the plasma of a patient with MCAD deficiency. C8 is octanoylcarnitine. Illustration provided by Jon Gangoiti of the University of California – San Diego.

For this test to be successful, it is sometimes necessary to administer some carnitine.

The pathology of MCAD deficiency is predominantly that of the liver [13, 17, 35] in which microvesicular and microvesicular deposits of fat are typical. Deposition of lipids has also been observed in the kidneys and heart [19]. Cerebral edema has been described in the neuropathology of MCAD deficiency, but this has rested on very little evidence. In the paper usually quoted [2], a two-year old, one of three patients reported, died and had cerebral edema and herniation on autopsy. Our teenager with MCAD deficiency [14] certainly had increased intracranial pressure, but she had hyperammonemia (235 μmol/l), a well-recognized cause of cerebral edema, and her encephalopathy resolved with the level of ammonia. Cerebral edema was also reported in two patients by Duran *et al.* [36]. In the series of 120 patients with MCAD deficiency, cerebral edema was recorded in 14 of 23 studied at autopsy [19]. Cerebral edema was also recorded by Bennett *et al.* [26] in an infant with MCAD deficiency who died suddenly in her sleep.

GENETICS AND PATHOGENESIS

MCAD deficiency is autosomal recessive [37–39]. The gene is on chromosome 1 and the nucleotide sequence of its cDNA has been established. A single mutation in which nucleotide 985 has been changed from A to G, leading to a lysine (K) to glutamic acid (E) change in residue 329 of the protein, accounts for virtually all of the patients identified prior to the addition of the disease to programs of newborn screening [36–38] (Figures 39.7 and 39.8). Among 172 patients, 80.2 percent were homozygous for A985G, and 17.4 percent were heterogyzous for this mutation [39]. Only 4 percent did not have this change on either allele. A second mutation was found in a patient with MCAD deficiency who was heterozygous for the A985G and a 4bp deletion [40]. Rapid screening is available for both mutations, which accounted for over 93 percent of all MCAD mutations in patients presenting with symptomatic disease. The common mutation leads to a high frequency of missplicing of mRNA [41], which would be expected to lead to variable phenotypic expression. Heterozygote detection and prenatal diagnosis can be carried out by testing for these mutations.

Identification of newborns in the United States with MCAD deficiency by MS/MS screening has yielded an incidence of 1 in 15,000 [42].

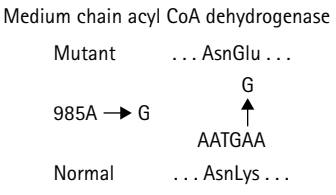

Figure 39.7 The mutation at nucleoide 985. The A to G change specifies a change from the basic lysine to the acidic glutamic acid.

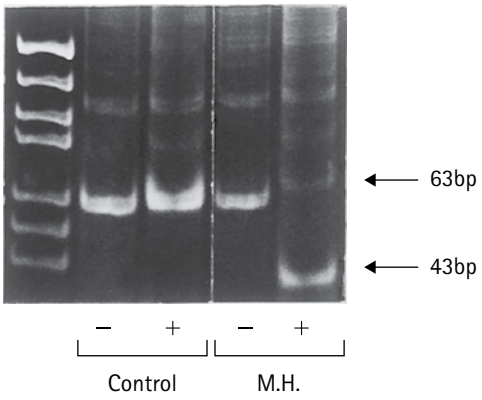

Figure 39.8 Detection of the common mutation in MCAD deficiency; autoradiography of DNA fragments after electrophoresis on a 3% agarose gel. This preparation was made by Dr. Karin Sege-Peterson with the method of Matsubara *et al.* [33], in which PCR with a mismatched primer permitted a restriction site in the mutant, but not in the normal. Thus, in the mutant, a 63 bp fragment contains the site which leads to a 43 bp fragment when treated with NcOI. In this illustration, – and + signify without and with NcOI; in the normal, there is no cleavage, while in the patient, M.H., the 43 bp fragment appears.

Mutation analysis revealed a lower incidence of the A985G mutation among those identified by newborn screening than had been observed in populations diagnosed after the onset of clinical illness. A previously unrecognized mutation, T199C, which had never been found in patients with clinical illness, appears to code for a mild phenotype. Expression of the recombinant Y42H protein coded for by T199C yielded about 80 percent of control activity and even more with chaperonin coexpression, indicating that the mutation interferes with protein folding but confers only mild interference with activity. The carrier frequency of this mutation appears to approximate 1 in 500. This mutation was never found in a sample of more than 90 patients identified by the presence of clinical illness. In this study, a mild acylcarnitine profile, as seen in patients heterozygous for A985G and T199C, had a C8 concentration of 0.5–2.0 μmol/l and a C8/C10 ratio of 2–4, while the severe profile, found in all the A985G homozygotes studied, had a C8 over 2 and a ratio of over 4. These data confirm the reliability of newborn screening for clinical MCAD deficiency. Some 11 other mutations identified were rare, including IVS 8 + G→ T, which changed a splice consensus, were associated with severe deficiency of the enzyme. In a family with A985G homozygosity, both the father and his son carried the mutation [43] (Figure 39.9).

Among infants identified by newborn screening, Zschocke *et al.* [44] found heterozygosity for the A985G mutation and Y67H in two patients, homozygosity for G267R in one, and S245L in two children of consanguineous parents. None of these patients had clinical disease up to six months of follow up at report. Urinary organic acids were normal in these infants, and their C8/C12 acylcarnitine ratios were lower than in patients with classic disease.

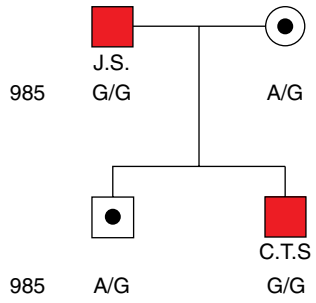

Figure 39.9 The pedigree of a family [43] in which a 12-month old admitted with hypoketotic hypoglycemia and the A985G mutation was found to have a father who was also homozygous for this mutation. The father had had two episodes of symptomatic hypoglycemia in infancy and had, therefore, studiously avoided fasting. The mother and a brother of the proband were heterozygous.

Enzyme assay showed higher activity than in classic patients.

The enzyme, MCAD, is one of three mitochondrial acyl CoA dehydrogenases (Table 39.1) that catalyze the initial steps in the β-oxidation of fatty acids (see Figure 39.1). Each is a flavin containing dehydrogenase that is specific for CoA esters of specific chain length. MCAD accepts fatty acyl CoAs in which the acid chain length is 6–12 carbons in length. The enzyme may be assayed in leukocytes, liver or cultured fibroblasts, as well as amniocytes. Immunochemical study of patients with the common mutation [45] revealed no evidence of CRM, and pulse chase labeling indicated that the enzyme is unstable.

The oxidation of fatty acids is not called upon in the production of energy until fasting has proceeded for some time. Glycogen stores suffice to provide carbohydrate for energy for 12 hours in most individuals. Thus, a history of hypoglycemia after a short fast implies a disorder of carbohydrate metabolism, while hypoglycemia after a prolonged fast implies a disorder of fatty acid oxidation. We have encountered exceptions to both rules but, in general, when we have subjected patients in remission with disorders of fatty oxidation to fasting under controlled conditions, hypoglycemia has seldom ensued before 16–18 hours (see Figure 39.5). This is consistent with the fact that the usual presentation is after seven months (median age 13.5 months [37]), usually concomitant with a first infectious illness that leads to anorexia or prolonged vomiting and its attendant fasting. It also explains the fact that, in some patients, the first episode occurs in a teenager [14] or adult [43]. The recognition of asymptomatic affected adults is consistent with the fact that some people never experience a fast longer than 16 hours. We expect that the incidence of normality will be high in those infants detected through routine screening.

In our experience, the exceptional patient who developed hypoglycemia after shorter periods of fasting, was a young infant who developed multiple episodes during infancy. The occurrence of SIDS is of course another exception to the expected course [46–48], and fatal neonatal presentation has been reported [6] in an infant with hypoglycemia and normal levels of free carnitine who had severe lipid cardiomyopathy at autopsy. GCMS of the liver in patients with SIDS has yielded cis-4 decenoic acid (C10:1) in each of four infants found to have had MCAD deficiency [47]. The prognosis for survival appears to be particularly bad for those with a neonatal presentation, although overall mortality in the first episode may be as high as 60 percent [46]. In patients surviving to diagnosis, the prognosis is good. Physical and intellectual development may be normal, although abnormal psychometric tests of development were surprisingly frequent in the survivors reported by Iafolla *et al.* [19, 20]. We expect that the incidence of normality will be high in those infants detected through routine screening such tests.

TREATMENT

The hallmark of treatment is the avoidance of fasting. Supplies of readily accepted and tolerated oral carbohydrate should be plentiful and accessible in the home. In a fragile infant, a supply of glucose-monogel®, or even parenteral glucagon, may be useful. In the presence of vomiting or anorexia that prevents oral intake, parenteral glucose is mandatory. Admission to hospital is prudent, but sometimes remission can be accomplished in the emergency room. Rates of administration and concentrations should be adequate to reverse hypoglycemia and maintain normoglycemia. It is not sufficient to start 5 percent glucose and relax; we have seen patients in whom symptomatic hypoglycemia developed under those circumstances. In long-term management, we have routinely employed supplemental cornstarch, at least for evening and night feedings. The initial dosage we have employed is 0.5 g/kg (1 tbsp = 8 g), usually working up to 1.0g/kg. In the fragile infant referred to above, 2.0 g/kg appeared to be helpful. Some reduction in the intake of fat appears prudent, but this does not need to be excessive.

Supplementation with carnitine is currently controversial, but why this is so appears difficult to understand. Patients are demonstrably deficient in free carnitine in virtually any circumstance in which they have lived undiagnosed past early infancy [49]. The very high urinary esterified carnitine and its major rise coupled with the specific increase in excretion of octanoylcarnitine and hexanoylcarnitine implies a detoxification function that should well be employed. During illness, octanoylcarnitine excretion increases dramatically when the patient is given IV carnitine [50]. Figure 39.5 illustrates considerably improved ketogenesis and an absence of symptoms despite fasting hypoglycemia in a patient treated for only a few days with carnitine. An absence of effect after three months of treatment with carnitine in a five-month old infant was reported because of the development of symptoms and hypoglycemia after 16.5 hours of controlled fasting [48]. However, the investigators permitted this

patient to fast only 12 hours prior to treatment, which did restore concentrations of carnitine in plasma to normal and markedly increased urinary carnitine excretion. Furthermore, the blood level of 3-hydroxybutyrate rose to 0.84 mmol/1, while prior to carnitine it failed to exceed 0.38 mmol/l. In a study of five symptom-free patients [51], acylglycine excretion exceeded acylcarnitine excretion by a factor of 70 to 1, but the amounts could not be increased by supplemental oral glycine. Supplemental carnitine increased acylcarnitine excretion six-fold and caused a 60 percent reduction in acylglycine excretion. In another study of monitored fasting [52], a patient tolerated a 12-hour fast after treatment, whereas before, 12 hours of fasting had induced a depressed sensorium and acidosis, as well as the expected accumulation of free fatty acids in the blood and dicarboxylic acids in the urine. We suspect that the failure to recognize a role for carnitine in treatment stems from the fact that once diagnosed, these patients do well if they avoid fasting. Most of our patients have not had a second episode requiring admission to hospital, but of course all of them have been treated with carnitine. An initial dose of 60–100 mg/kg is useful. During acute illness, we use 200–300 mg/kg IV. Treatment with 50–150 mg riboflavin/day was reported [53] to increase the activity of MCAD in lymphocytes of five patients with MCAD deficiency. Increases were very small in four, but a major increase in one patient, who began with 19 percent of control activity, suggests that supplementation may be a useful adjunct.

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40

Very long-chain acyl-CoA dehydrogenase deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, hepatomegaly, cardiomyopathy and myopathy, rhabdomyolysis, elevated creatinine kinase, lipid infiltration of liver and muscle, and defective activity of very long-chain acylCoA dehydrogenase (VLCAD).

INTRODUCTION

Very long-chain acyl-CoA dehydrogenase (VLCAD) is bound to the inner mitochondrial membrane. It was first delineated in 1992 [1] as catalyzing the dehydrogenation of acylCoA esters of 14 to 20 carbon length in the first step of mitochondrial fatty acid oxidation (Figure 40.1). Within one year, there were three reports [2–4] of patients with deficiency of VLCAD, including some who had been previously reported as having long-chain acyl-CoA dehydrogenase (LCAD) deficiency [5]. It is now recognized that all of the patients initially described with LCAD deficiency [6] appear, in retrospect, to have had defects in VLCAD [5]; and the LCAD enzyme catalyzes the specific oxidation of branched long-chain acylCoAs. The usual assay with palmitoyl CoA as substrate in the presence of electron transfer flavoprotein (ETF) would register deficiency of activity if either LCAD or VLCAD was deficient. The distinction can be made by immunochemical or genetic analyses.

VLCAD deficiency is relatively common; the incidence is 1:85,000 [7]. It is the most common disorder of fatty acid oxidation in the Saudi population. There were 12 patients among the series of 107 disorders of fatty acid oxidation in the Paris experience of Saudubray *et al.* [8]. The *ACADVL* gene encoding for VLCAD has been isolated [9] and found to contain 20 exons; it is situated on chromosome 17p13.1 [10–13]. Heterogenous mutations (over 150 affecting all 20 exons) have been identified [13–17].

CLINICAL ABNORMALITIES

Impairment of very long-chain acyl-CoA dehydrogenation can result in severe organ dysfunction, especially of the heart, liver, and skeletal muscle. The disease may present in the first days of life. One patient [2] had metabolic acidosis at two days of age. The blood level of creatinine kinase (CK) was 3684 U/L, and he had impressive dicarboxylic aciduria. A sibling died suddenly without cardiac abnormalities and had massive fatty infiltration in the liver. In six families

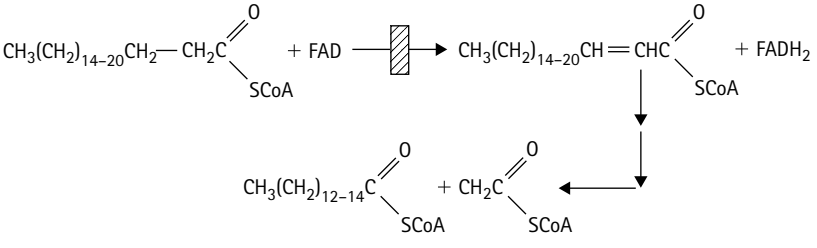

Figure 40.1 The VLCAD reaction. Following the formation of the enol product, the three successive reactions catalyzed by enoyl CoA hydratase, 3-hydroxyacyl CoA dehydrogenase, and 3-ketoacyl CoA hydratase yield ultimately acetyl CoA.

of 11 patients with VLCAD deficiency, there were eight instances of sudden infant death syndrome (SIDS) or unexplained death [5].

By now, three overlapping clinical phenotypes of VLCAD deficiency can be delineated [17, 18]: the severe form with cardiac involvement and the milder form with hypoglycemia as in medium-chain acylCoA dehydrogenase (MCAD) deficiency, both with early onset and hypoketotic hypoglycemia (Figures 40.2 and 40.3), and there really is a third phenotype characterized by episodic rhabdomyolysis and myoglobinuria [19]. There is some merging of these clinical forms, but distinction may be useful because it tends to correlate with amounts of residual enzyme activity [5]. In addition, extended newborn screening is identifying an increasing number of individuals who are, as yet, completely asymptomatic.

In a major series of 54 patients about half suffered from the severe, early onset presentation within the first three days of life, cardiomyopathy in 92 percent, hepatomegaly in 80 percent, and early death in 80 percent [17]. Nevertheless, diagnosis and treatment are consistent with survival, reversal of cardiomyopathy, and relative health at time of report [4, 5, 20]. One patient who had hypoketotic hypoglycemia and cardiomyopathy died at eight days of age of a penetrating duodenal ulcer and peritonitis [21].

Neonatal presentations include lethargy, tachypnea, or seizures, and hypoglycemia; metabolic acidosis or arrhythmia may be found. This is followed by decompensation and evidence of hypertrophic cardiopathy. There may be pericardial effusion. Approximately 50 percent of patients have died within two months of initial symptomatology [17, 22].

Some patients have had a more typical fatty acid oxidation presentation with fasting intolerance and acute hypoketotic hypoglycemia, usually presenting at the first intercurrent infection, and followed by episodic hypoglycemia. Cardiomyopathy is less common in this group. Patients or previous siblings have been diagnosed as having Reye syndrome [23]. Plasma ammonia may be elevated. Uric acid and CK are also high during attacks. An interesting variation on this theme was a patient who presented at two years with hypoglycemia and encephalopathy (glucose 1.7 mmol/L) and acidosis resulting from massive ketosis [24]. Because of this, a disorder of fatty acid oxidation was not considered, although the CK was 5373 U/L. The diagnosis was made when the acylcarnitine profile revealed elevated tetradecanoylcarnitine and was confirmed by enzyme analysis. As these patients become older, metabolic decompensations subside, but muscle pain becomes a problem as they undertake more exercise. Symptoms may change from episodes of hypoketotic hypoglycemia to the muscular form of the disease.

A third presentation is reminiscent of carnitine palmitoyl transferase (CPT) II with episodic muscle pains, rhabdomyolysis, and myoglobinuria [19, 25]. One 28-year-old woman experienced her first symptoms, which were

Figure 40.2 DS: A 16-month-old girl with VLCAD deficiency. At 12 months of age, she was admitted in shock with a blood sugar of 0 and a seizure. She had modest dicarboxylic aciduria. Tandem mass spectrometry revealed elevated long-chain acyl carnitine esters. She was thought to have hypopituitarism and hypothyroidism on the basis of abnormal test results at 12 months, but by six years these abnormalities disappeared, and she has grown well without replacement therapy since that time.

Figure 40.3 AG: A two-year-old girl with VLCAD deficiency. Diagnosis was made at two days of age; a previous sibling had died of a disorder of fatty acid oxidation. Acylcarnitine profile revealed elevated C14:1, C16, and C18:1. She had bilateral inverted nipples.

induced by exercise, at 19 years [25]. Levels of CK in the blood were very high.

Plasma free carnitine may be normal or low. Urinary carnitine may be low, especially at times of acute illness, as long-chain acylcarnitine esters are not well excreted by the kidney. The accumulation in plasma of C14:1carnitine esters is the most specific finding on acylcarnitine profile (Figure 40.4), and may be detected in blood spots in newborn screening [26]. Levels of C14:1, C14, C16, and C18:1 may be elevated, and the ratios of C14:1 to C14 or C14:1 to C18:1 may be particularly useful in diagnosis [26, 27]. C14:1/C2 may be a more sensitive marker than C14:1, but could rise the risk of overdiagnosis [28]. Incubation of fibroblasts with ^{13}C-labeled palmitate revealed accumulation in C16; in this system C14:1 was not elevated [29].

Organic acid analysis of the urine may reveal dicarboxylic aciduria. Unfortunately, during interepisode periods of health, when many diagnostic work ups occur, organic acid analysis is normal. During episodes, there is medium-chain, as well as long-chain dicarboxylic aciduria indicating the functioning of peroxisomal β- and ω-oxidation.

Pathologic examination reveals hepatic steatosis and deposits of lipid in cardiac and skeletal muscle. Mitochondrial appearance may be abnormal [30]. Peroxisomes may be enlarged.

A C14:1-carnitine level >1 μmol/L correlates very well with enzyme- or mutation-proven disease [31], whereas concentrations below 1 μmol/L do not allow a clear discrimination among affected patients, carriers, and healthy individuals. Cut-off levels of <0.25 μmol/L yield a number of false positives. Thus, confirmatory assays are necessary. A repeat screen or a plasma acylcarnitine profile is not that useful, because a normal value may be found in patients with the disease. Oxidation of palmitoyl CoA in lymphocytes with high performance liquid chromatography (HPLC) separation of the products, particularly 2-hexadecenoyl CoA [31, 32], has been useful in making a definitive diagnosis. Mutational analysis has become a common approach to this problem, but finding a single mutation does not prove heterozygosity because this has been found in the enzyme-proven patient [31].

GENETICS AND PATHOGENESIS

VLCAD deficiency is transmitted in an autosomal recessive fashion. The enzyme was purified from rat liver mitochondria [1]. It is loosely bound to the inner mitochondrial membrane and requires ETF as the electron receptor. It is unique among the acyl-CoA dehydrogenases in its size, structure, and intramitochondrial distribution. Unlike the other mitochondrial acyl CoA dehydrogenases, it is a 154 kD dimer of a 70 kd subunit. It does not crossreact with antibodies against LCAD or other acyl CoA dehydrogenases. Its activity is greatest against C16, palmitoyl CoA, and activity is ten times that of LCAD. Deficiency of VLCAD may be proven in cultured fibroblasts. Antibody against VLCAD is reduced by 66–75 percent, and this may be demonstrated by Western blot analysis.

When antibody to LCAD became available, nine cell lines previously thought to be deficient in LCAD were tested and found to have normal immunoreactive LCAD protein [33], and testing via immunoblot analysis against VLCAD revealed them all to be VLCAD-deficient. Low VLCAD activity was also demonstrated by testing for enzyme activity in the presence of anti-LCAD antibody which did not alter activity [3].

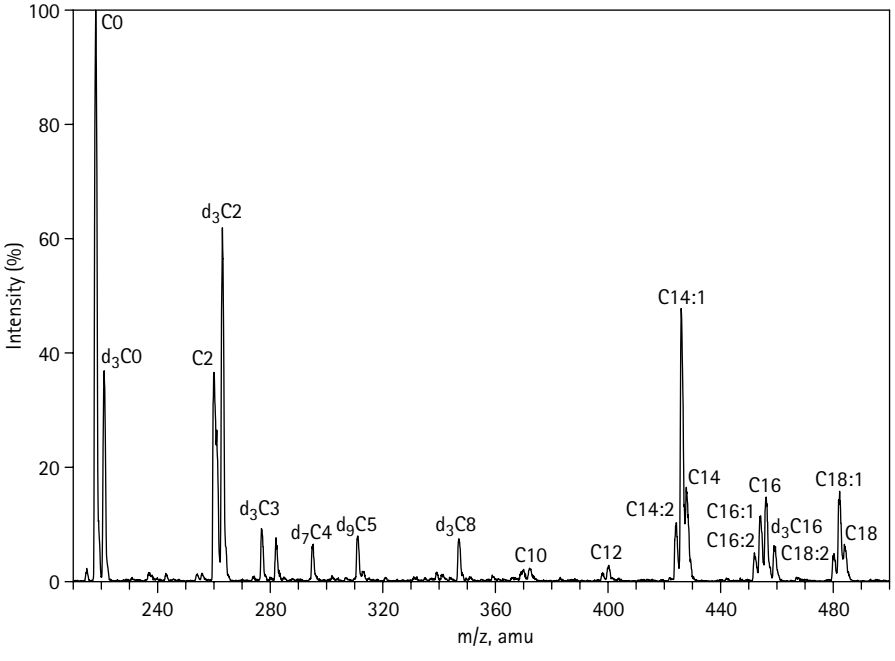

Figure 40.4 Acylcarnitine profile of the blood plasma of a patient with VLCAD deficiency. The patient was being treated with carnitine, and the C0 was elevated. The key compound was C14:1 acylcarnitine, but all of the long-chain acylcarnitine esters were elevated. Illustration provided by Jon Gangoiti of UCSD.

Rapid indication of the diagnosis has been reported by the study of β-oxidation and VLCAD in lymphocytes [31] and the method has been adapted for prenatal diagnosis of chorionic villus material. Prenatal diagnosis has also been made by assay of the dehydrogenation of palmitoyl CoA in amniocytes [34].

In vitro studies of the incubation of fibroblasts with deuterium-labeled palmitate and carnitine followed by assay of the pattern of enrichment of acylcarnitines [5] has been reported to correlate well with the different phenotypes of severe cardiomyopathy and those without cardiomyopathy. The ratio of deuterated C16 to deuterated C12 has been discriminatory. Identification of 13 patients with myopathic phenotypes was made by immunoblot chemical of biopsied muscle [35].

The gene for VLCAD has been cloned and sequenced [16]. Over 150 mutations have been reported, and genotype/phenotype correlations have been established in a study of 32 unrelated patients [17]. In general, patients with the more severe phenotype had alleles coding for truncated proteins or proteins lacking amino acids consistent with predicted null activity [17, 19, 21, 22], whereas those with the milder phenotypes had alleles with missense mutations [17]. In a 14-year-old patient with recurrent myalgia and CK elevation with moderate exercise, the attenuated phenotype was associated with mutations A416T and R450H each of which expressed as temperature-sensitive enzymes [36]. The woman whose symptoms began at 19 years had two missense mutations (G145C/R375W) [19]. An interesting S583W mutation demonstrated that association of the mature VLCAD protein with the inner mitochondrial membrane is required for activity, because the protein is imported normally [14]. Of 37 patients, only one mutation was found in seven [37], despite sequencing of all of the exons. A high incidence of cardiomyopathy provides caution about concluding that the finding of only one mutation indicates heterozygosity.

TREATMENT

Treatment aimed at the hypoglycemia emphasizes the avoidance of fasting and prompt intervention with parenteral glucose-containing solutions when fasting is unavoidable. Guidelines in the diagnosis and management of long-chain fatty oxidation defects have been published [38, 39]. We provide parents with a letter, which can be produced at a medical facility, stressing the necessity of intravenous (IV) glucose under such conditions, even if an initial glucose level is normal. Fasting attendant upon surgery and anesthesia, particularly for minor procedures, may be particularly dangerous [40]. Early preparation with IV glucose prevents problems. Cornstarch supplementation may be useful (1.5–2 g/kg at bedtime). In patients with severe forms, continuous nocturnal intragastric feeding is necessary because even small persistent lipolysis can result in accumulation of toxic acylcarnitines. Adolescents and adults should never fast for more than 12 hours overnight. Medium-chain triglyceride (MCT) appears to be therapeutic and surviving patients have done well after initiation of MCT supplementation. A diet low in fat (5–10 percent of calories in long-chain triglycerides) supplemented with MCT (30 percent of total calories or 85–90 percent of the calories from fat) was credited with reversal of hypertrophic cardiomyopathy [20]; the patient also received 50–100 g/kg of carnitine. Similar resolution of cardiomyopathy was reported in a boy treated with a similar regimen; he and his prenatally diagnosed sister had normal cardiac and developmental function at follow up [30]. In the course of treatment, deficiency of ω-6 fatty acids, such as DHA and arachidonic acids, has been reported [41], but neither patient had any symptoms of deficiency; specifically there was no pigmentary retinopathy as in LCHAD deficiency (Chapter 41), and the levels do not seem especially low to those of us monitoring very low-fat diets, such as those employed for lipoprotein lipase deficiency (Chapter 86). Spiekerkoetter reported that in asymptomatic VLCAD deficiency, a fat-reduced diet may not be necessary, whereas in later infancy and adolescence, strenuous physical exercise may require additional energy from medium-chain fat [42].

Carnitine therapy has become controversial in this as well as other long-chain fatty acid oxidation disorders, particularly because of the fear that long-chain acylcarnitines would accumulate and provoke arrhythmias [43]. Also, long-chain acylcarnitine esters have been reported to promote ischemic damage or abnormal post-ischemic function in experimental animals [43, 44], and their prevention by inhibitors of acylcarnitine formation, but another study found no effect of a CPT I inhibitor and obtained evidence that the ischemic damage resulted from the fatty acids [45]. In VLCAD-deficient mice, carnitine supplementation increased the content of acylcarnitine esters in muscle and liver without replenishing free carnitine [46]. Incubation of hepatic cells with carnitine decreased their viability. Treatment with carnitine was reported to ameliorate recurrent myoglobinuria in an 11-year-old patient with VLCAD deficiency [47], but to be without effect in another patient [19].

In fibroblasts of patients with CPT II deficiency, pharmacologic enhancement of enzyme activity *in vitro* has been demonstrated with benzafibrate, an agonist of the peroxisome proliferator activated receptors (PPARs) [48]. This has been extrapolated to VLCAD fibroblasts with similar enhancement of oxidation capacity *in vitro* [49]. Interestingly, not all genotypes of VLCAD deficiency or mitochondrial trifunctional protein deficiency may be improved *in vitro* with bezafibrate treatment [49, 50]. In a randomized double-blind crossover study, bezafibrate treatment of patients with VLCAD deficiency did not improve clinical symptoms during exercise [51].

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41

Long-chain L-3-hydroxyacyl-CoA dehydrogenase – (trifunctional protein) deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, episodic rhabdomyolysis, hypotonia, cardiomyopathy, hepatic disease, peripheral neuropathy, pigmentary retinopathy, 3-hydroxydicarboxylic aciduria, elevation of characteristic long-chain acylcarnitines and defective activity of the trifunctional protein or isolated deficiency of the long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) subunit or the long-chain ketothiolase (LCKAT) subunit. Maternal acute fatty liver of pregnancy during carriage of a fetus with LCHAD deficiency.

INTRODUCTION

Long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency was first reported [1, 2] in 1983 in a boy who had many attacks of hypoketotic hypoglycemia starting at nine months of age, had hypotonia and cardiomyopathy, and went on to develop massive hepatic necrosis, and died at 19 months. There was long-chain acylcarnitine accumulation in plasma and 3-hydroxydicarboxylic aciduria. The activity of LCHAD was demonstrated to be defective in an assay in which 3-ketopalmitoyl CoA was the substrate.

The LCHAD enzyme is a component of the trifunctional protein (TFP) bound to the inner mitochondrial membrane [3, 4]. The protein is an octamer with two distinct α and β subunits coded for by the *HADHA* and *HADHB* genes. Its three activities are long-chain 2-enoylCoA hydratase, LCHAD, and long-chain 3-oxoacylCoA thiolase (LCKAT). The first two enzymatic steps (dehydrogenase and hydratase) reside in the α-subunit and the thiolase activity in the β-subunit. LCHAD activity against 3-hydroxyacyl CoA substrates is optimal for compounds of C12–C16 chain length, in contrast to the short-chain-3-hydroacylCoA dehydrogenase (SCHAD), where specificity is optimal at C6. LCHAD action is highest at C16 and inactive at C4. The thiolase and enoyl hydrolase activities also have long-chain specificities. The LCHAD enzyme catalyzes the reversible dehydration of the 3-hydroxy group to a 3-keto group, and nicotine adenine dinucleotide (NAD) is the hydrogen acceptor (Figure 41.1). Patients with LCHAD deficiency may be deficient in LCHAD activity specifically, or may be deficient in all three activities of the TFP. The genes for the α- and β-subunits have been cloned [5]. The α-cDNA codes for an 82,598 Da precursor of a mature 78,969 Da protein. In mitochondrial trifunctional protein (MTP) deficiency, all three activities, dehydrogenase, hydratase, and this thiolase are deficient [6]. Mutational analysis has revealed a number of distinct mutations including one that appears to be common, a G1528C point mutation in the dehydrogenase coding region that changes a glutamic acid to a glutamine [6, 7]. Isolated deficiency of LCHAD

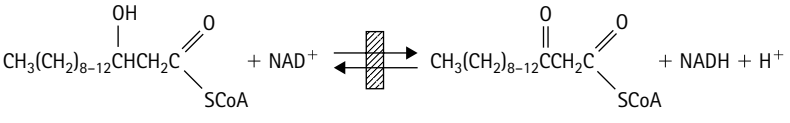

Figure 41.1 The reaction catalyzed by long-chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD). The product is then involved in the 3-ketothiolase reaction in which the bond is cleaved and acetyl CoA split off, yielding a fatty acid CoA ester of two less carbons.

is coded for by specific mutations in the *HADHA* gene, especially the common G1528C point mutation [8]. Isolated deficiency of LCKAT activity has been reported once caused by a mutation (F431S) in the *HADHB* gene [9]. Generalized TFP deficiency can result from heterogeneous mutations in either the alpha-subunit or the beta-subunit of the TFP.

CLINICAL ABNORMALITIES

Patients with LCHAD deficiency usually present in late infancy with the typical clinical picture of a disorder of fatty acid oxidation of which the hallmark feature is acute hypoketotic hypoglycemia (Figures 41.2 and 41.3). These episodes often begin late in the first year of life, with the first long fast, usually caused by an intercurrent infection

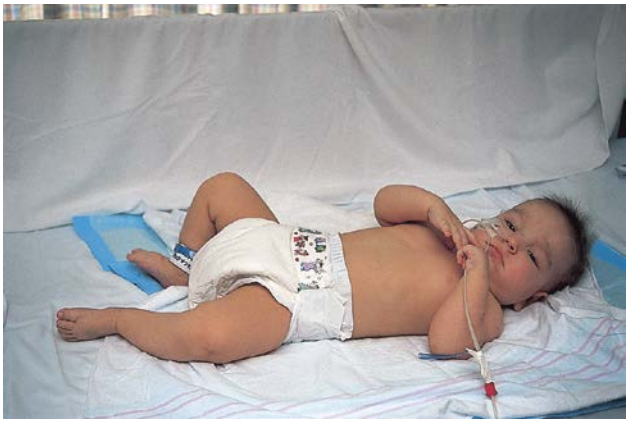

Figure 41.2 LJ: A nine-month-old boy with long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency. He had a number of episodes of hypoglycemia starting at five months of age, when he was found to have hepatomegaly and hepatic steatosis. Fasting and loading with long-chain triglycerides led to hypoglycemia, while medium-chain triglyceride (MCT) loading was uneventful. Later, he had an episode of myoglobinuria and massive elevation of creatinine phosphokinase.

Figure 41.3 The feeding tube reflected the need, prior to referral, for virtually continuous feeding to maintain normoglycemia.

illness and ushered in with vomiting [8, 10–13]. Many have been diagnosed as having Reye syndrome. Mean age of onset in 50 patients was between five and eight months with a range from two days to 21 months [11]. The disease may be a cause of sudden infant death, even neonatal [14, 15]. With prompt diagnosis and treatment, acute neonatal cardiorespiratory arrest may yield to resuscitation and a favorable prognosis [16]. During the acute episode, levels of creatine phosphokinase (CK) and uric acid are elevated [17].

The acute episode may begin with a seizure; the electroencephalogram (EEG) may be abnormal. Most patients are hypotonic at least in infancy. Patients may be difficult to feed, and gavage feeding may be required [14]. Some may display failure to thrive.

Later episodes are often ushered in with pains in the legs. Rhabdomyolysis leads to myoglobinuria [18]. Patients may first present as adults with exercise-induced muscle pains and rhabdomyolysis. Levels of CK may be very high (15,000–165,000 IU). Examination may reveal profound weakness, little movement, and the assumption of a frog-leg position. Patients can present with chronic progressive polyneuropathy and myopathy without hepatic or cardiac involvement [19].

Some patients with myopathic presentations have had rapidly fatal cardiomyopathy in infancy [20, 21]. Acute life-threatening cardiac episodes may be followed by tetraparesis [21]. Such patients may, or may not have had earlier episodes of hypoketotic hypoglycemia. Examination of the heart may reveal cardiomegaly, poor heart sounds, and gallop rhythm. The electrocardiogram reveals sinus tachycardia, a long QT, ventricular tachycardia, or a long left ventricular hypertrophy [21]. Echocardiography may reveal dilatation and poor contractility [20]. Pericardial effusion has been reported, as well as tamponade [13, 21]. Others have had a more indolent, myopathic presentation in which ventricular hypertrophy is found on echocardiography or electrocardiography in the absence of symptoms [14].

Hepatic dysfunction is another characteristic of the disease [1, 2, 11, 12, 20]. Most patients have hepatomegaly [12, 20]. Some have had acute cholestatic jaundice as neonates, and this may be transient [12]. The other end of the scale is massive total hepatic necrosis in infancy [1]. Jaundice may develop in infancy along with elevation in the blood levels of transaminases. Ultrasound or other imaging may indicate fatty infiltration of the liver. Biopsy reveals accumulation of fat and fibrosis.

An unusual result of hepatic disease was cholestatic jaundice and impaired 25-hydroxylation of vitamin D leading to hypocalcemia and a presentation at two months of age with a tonic-clonic seizure [22]. Several authors reported patients with LCHAD deficiency or TFP deficiency with hypoparathyroidism [23].

A more unusual complication is the acute fatty liver of pregnancy in a mother carrying a fetus with LCHAD deficiency [22]. Heterozygous mothers may have pre-eclampsia and urinary protein, or the hypertension, elevated liver enzymes and low platelets (HELLP) syndrome or acute

fatty liver of pregnancy (AFLP). As many as approximately 20 percent of pregnancies at risk may be complicated by one of these problems [11]. Among women who have AFLP, 10 to 25% will carry a fetus with LCHAD deficiency. LCHAD deficiency is much less common in patients with HELLP, occurring in less than 1 percent of these pregnancies.

It has increasingly been recognized that pigmentary retinopathy is a potential complication of LCHAD deficiency [10, 11, 24–26]. This may occur in as many as 70 percent of patients, but as yet the true incidence is unclear, as visual problems are progressive, and few patients have been followed for very long. In a series of 28 children, a pattern of ophthalmologic progression emerged [24]. Of 15 patients who died at ages from three to 14 months, vision had been normal for age. In the oldest survivors (16 and 31 years), visual loss was progressive. In 11 children, granular retinal pigment was seen at four months to five years. The two long-term patients had progressive atrophy of the choroid and retina, axial myopia, and scotomata. All four longer-surviving patients had lenticular opacities. The electroretinogram deteriorated during the final decade and became unrecordable in the oldest patient. Posterior staphylomas were seen in the two oldest patients.

Another clinical abnormality unusual in disorders of fatty acid oxidation that has been observed with time in LCHAD deficiency is peripheral neuropathy [18, 24]. By adolescence, neuropathy and retinopathy may be the major clinical problems [24]. Deep tendon reflexes may be absent even in infancy [25]. The patient may toe-walk and display an equinus deformity. Extensor plantar responses have been reported [25]. In one patient, mild peripheral neuropathy of adult onset was the only clinical abnormality [27].

Intelligence in these patients has usually been normal, but of course prolonged hypoglycemia always carries a risk of injury to the central nervous system, and a number of patients have had impaired mental development and/or had a seizure disorder [11]. Interestingly, six of 10 patients had anemia and thrombocytopenia. Mortality has been as high as 38 percent [11]. Morbidity in surviving patients has also been high [11], especially acute muscle problems and episodic metabolic derangement [11]. On the other hand, it was notable that all who died did so within three months of diagnosis, either in the first episode or due to progressive disease resulting in cardiorespiratory failure. In those surviving, none had cardiomyopathy, and their clinical condition was good despite recurrent muscle problems or diminished visual acuity.

Clinical manifestations of MTP deficiency and isolated LCHAD deficiency are generally indistinguishable, but in one series [12], 42 percent of patients with MTP had rapidly progressive deterioration; eight of the nine died of cardiac disease within two months. The ninth died of hepatic failure at four weeks. Two patients diagnosed prenatally died despite treatment; one had hydrops fetalis. Two pregnancies were complicated by HELLP syndrome. Among five Japanese patients with MTP deficiency [28], two had onset within the first days of life with lactic acidemia, hypoglycemia, and hyperammonemia. They died shortly of cardiac arrest. Two had hepatic presentations and rhabdomyolysis in late infancy. Another had an onset at 15 years of muscle pain, weakness, and rhabdomyolysis. Isolated LKAT deficiency was described in a newborn with lactic acidemia, pulmonary edema, and cardiomyopathy. He developed acute heart failure and died at six weeks of age [9].

The clinical chemistry in the acute illness may reveal hyperammonemia (68–400 mmol/L). This, with the hypoglycemia, hepatomegaly and elevation of transaminases is what has led to a diagnosis of Reye syndrome. The CK is elevated and so is the level of uric acid [17]. Lactic acidemia may accompany the acute episode, or there may be persistent lactic acidemia [1, 14, 18, 25]. Fatal neonatal lactic acidemia has been reported [28]. Carnitine is low, especially free carnitine. Free fatty acids are increased, and the ratio of free fatty acids to 3-hydroxybutyrate is particularly high. With hepatic dysfunction, there may be hyperbilirubinemia.

Pathologic examination has generally revealed microvesicular and macrovesicular accumulation of fat in the liver, skeletal muscle, and heart, but necrotic myopathy without accumulation of lipid has also been described [25] as has a predominance of type 1, slow oxidative muscle fibers. Hepatic cirrhosis has also been observed. Electron microscopy has revealed condensation of mitochondrial matrix and widening of crystal spaces [20, 29].

The diagnosis is most often suggested by the findings of large amounts of 3-hydroxydicarboxylic acids in the urine, or by the determination of the acylcarnitine profile in the blood. On gas chromatography-mass spectrometry (GCMS) organic acid analysis of the urine, the key compounds are hydroxy acids of up to 14 carbons [30], but medium-chain dicarboxylic and 3-hydroxydicarboxylic acids are also found [2, 29]. Quantification in organic acid analysis is important in this condition as in others, for 3-hydroxydecanedioic acid and other dicarboxylic acids may be found in the urine in elevated amounts in ketosis, but in smaller quantity than in LCHAD deficiency [31]. Any of these abnormalities may become normal during an interim period of health between acute episodes. We have followed a patient in whom 3-hydroxyadipic acid is the only organic acid marker of the disease, even at times of acute rhabdomyolysis. This compound may be elevated by ketosis, but of course patients with this disease do not become ketotic. Assessment of the acylcarnitine profile of the plasma should reveal 3-hydroxyacid derivatives of the C16, C18, and C18:1 species (Figure 41.4) [32]. In addition, the 3-hydroxyacylcarnitines of C14 and C14:1, are found [33], as well as the long-chain acylcarnitines of C12, C14:1, C14, C16, C18:2, and C18:1. Over 85 percent of patients could be identified by elevation of hydrox-C18:1 over the 95th percentile of controls in combination with an elevation of two other long-chain species, hydroxy-C(14)-hydroxy-C(14:1), or hydroxy-C(16:1). High levels of endogenous long-chain acylcarnitines in erythrocytes make blood spots much less reliable than plasma. The acylcarnitine profiles of total TFP

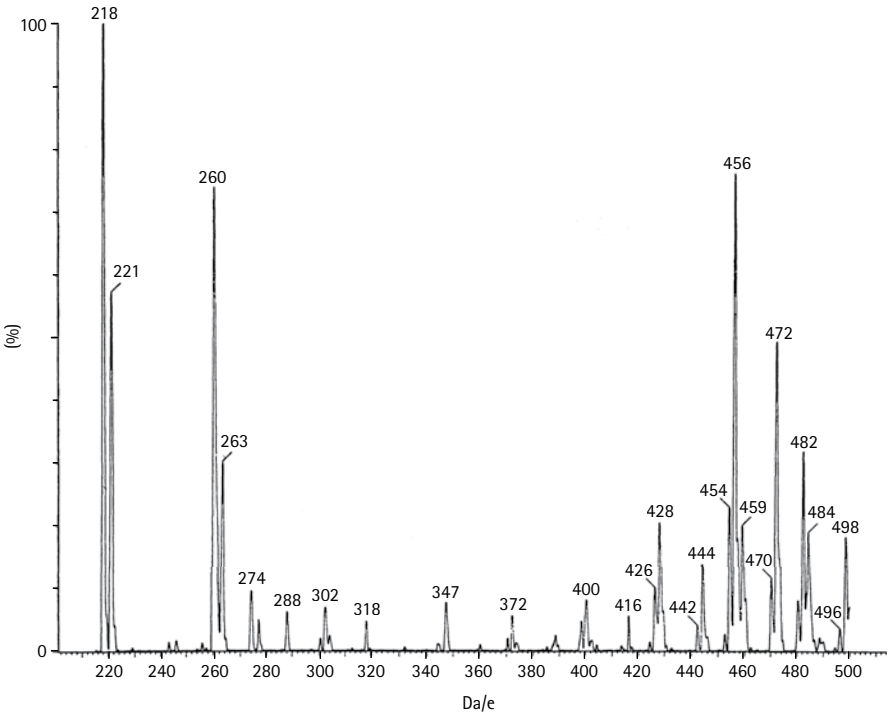

Figure 41.4 Acylcarnitine profile of the blood plasma of a patient with long-chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency. Key compounds were the 3-hydroxyl derivatives of C14, C16, and C18. (Illustration provided by Jon Gangoiti of UCSD.)

deficiency and isolated LCHAD and LKAT deficiencies are indistinguishable. Oral loading with 3-phenylpropionate leads to the excretion of 3-hydroxyphenylpropionate, indicating the site of the defect [15].

GENETICS AND PATHOGENESIS

LCHAD deficiency is transmitted as an autosomal recessive trait. The molecular defect is in the mitochondrial trifunctional protein, which contains activities of LCHAD, 2-enoylCoA hydratase, and 3-oxoacylCoA thiolase. It differs from the trifunctional enzyme found in peroxisomes in structure and function [3], and is capable by itself of catalyzing the three sequential steps of β-oxidation. In some patients, there is defective activity of all three activities of the protein [27], but in most of the patients, deficiency is isolated to LCHAD. Experience with newborn screening is now becoming available from an increasing number of programs worldwide [34]. The spectrum of disorders differs widely between ethnic groups. Incidence calculations from reports from Australia, Germany, and the United States of a total of 5,256,999 newborns give a combined incidence of approximately 1:250,000/1:750,000 newborns for LCAHD/TFP deficiency. Isolated deficiency of the thiolase has only been reported in a single child [9].

The diagnosis may be confirmed by study of the oxidation of ^{14}C-labeled myristic (C14:0) and palmitic (C16:0) acids by lymphocytes [35] or fibroblasts, or by mutational analysis [12]. Acylcarnitine profiling with ^{13}C-labeled or ^{14}C-labeled substrate is not different in total TFP deficiency and isolated LCHAD or LKAT deficiencies [9]. The enzyme has usually been measured in fibroblasts in the reverse direction, with 3-oxopalmitoylCoA as substrate and measurement of the decrease in absorbance at 340 nm of the NADH electron donor. In some patients, activity is undetectable [18, 36], but since the SCHAD enzyme has some activity against longer fatty acids, activity is usually about 15–35 percent of control [2, 13, 18]. Assay in the presence of antibody against the SCHAD protein generally gives LCHAD activity values less than 10 percent of control [2, 13, 18]. Enzyme activity of LKAT was measured with 3-ketopalmitoylCoA as substrate [9]. In rare instances, cross-reacting material (CRM) for TFP is virtually undetectable, and activity of the three enzymes is deficient but, in most instances, immunoreactive MTP is normal and activity of only LCHAD is deficient [37]. Indication of the diagnosis has also been made by incubation of fibroblasts with palmitate and analysis of the medium for free 3-hydroxyacids [38]. Levels of 3-OHC14 and 3-OHC16 were increased 11- and 14-fold.

Intermediate activity in fibroblasts has been consistent with heterozygosity. Prenatal diagnosis can be made by enzyme analysis and by mutational analysis [39].

The genes for both proteins have been cloned and localized to chromosome 2p23.3, and the common mutation in the α-subunit has been identified [7]. This G1528C mutation changes the glutamate 510 to glutamine. A simple polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) method for the detection of this mutation simplifies diagnosis and carrier detection [37]. Approximately 87 percent of chromosomes of patients with LCHAD deficiency have been found to carry the G1528C mutation [7, 11, 37]. This mutation has frequently been found in infants of mothers with acute fatty liver of pregnancy [22, 39, 40]. Expression studies indicated that the mutation induces loss of LCHAD activity [7]. Other

mutations have been detected, usually in compound with G1528C. These include C1132T, which changes glutamine 342 to stop [40]. The mutation has also been designated at amino acid 474 (E474Q) [40]. Homozygosity for G1528C has been reported to lead to severe disease and death in early infancy [11, 16]. An infant with neonatal hypoglycemia and death in infancy had two different splice-site mutations following exon 3 [41]. An infant whose mother had acute fatty liver of pregnancy was a compound of C1678T, which converted arginine 524 to stop and TFP deficiency with the common LCHAD mutation [42]. The gene for the β subunit has been localized also to chromosome 2p23 [43]. Patients with other than the G1528C mutations are among those with complete MTP deficiency and cardiomyopathy or neuropathy. In the patient with isolated LKAT deficiency, two mutations were found in the *HADHB* gene, c.185G>A (p.R62H) in exon 4 and c.1292T>C (F431S) in exon 15 [9].

In two unrelated patients, mutations were found for the first time in the *HADHB* gene [44]. In a French cohort of 52 patients with mitochondrial trifunctional protein deficiency, the majority of identified mutations generated premature termination codons resulting in nonsense mRNA-mediated decay [6]. It appears that both normal α and β are important for stabilization of the trifunctional protein. Lethal disease was reported in a case of uniparental disomy of chromosome 2 leading to homozygous mutation in *HADHA* [45]. A knock-out mouse lacking the α and β subunits of MTP has neonatal hypoglycemia and sudden death [46].

The lactic acidemia observed so regularly in this disease may result from inhibition by accumulated long-chain acyl CoA esters of the pyruvate dehydrogenase complex [47] or mitochondrial carriers, changing NADH/NAD ratios, or oxidative phosphorylation.

TREATMENT

Guidelines in the diagnosis and management of long-chain fatty oxidation defects have been published [48, 49]. The avoidance of fasting is important in the management as with all patients with disorders of fatty acid oxidation, including LCHAD deficiency. Because patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency seem to have an increased lipolysis in comparison to healthy subjects, the avoidance of fasting in these patients is of utmost importance [50]. Small infants need continuous enteral feeding or frequent meals (every 4 hours) in daytime and continuous nocturnal enteral feeding. Preschool children continue to need frequent meals during daytime (three meals and three intermeal snacks including one at bedtime) as well as uncooked cornstarch (1.5 to 2 g/kg) at night. The addition of medium-chain triglyceride (MCT) to the regimen has been reported to be therapeutic [1, 13, 14]. Dosage has been 1.5 g/kg. Treatment with MCT was followed by improvement almost to normal in dicarboxylic aciduria, as well as a return to normal of the plasma level of long-chain acyl carnitines [14]. The patients should also get a multivitamin and mineral supplement that includes all of the fat-soluble vitamins. Finally, the diet should be supplemented with vegetable or walnut oil as part of the 10 percent total long-chain fatty acids intake to provide essential fatty acids. Carnitine therapy restored to normal the level of free carnitine in plasma, but increased the concentrations of long-chain acyl carnitines. Although many patients have improved, not all have. Peripheral neuropathy and retinopathy do not appear to benefit from MCT and dietary treatment.

Carnitine has been employed in doses approximating 50–100 mg/kg per day. Its use has become debatable because of concern that long-chain acyl carnitine esters may be toxic, and some reports have suggested that carnitine-treated patients have done worse [8, 13, 20]. However, in the largest study, approximately half of the patients were treated with carnitine, and no ill effect could be demonstrated [11]. In our view, the use of 25 mg/kg per day carnitine in this disease makes sense. Riboflavin has been given in doses of 75–100 mg/day with no benefit.

Dietary restriction of long-chain fats in this disorder appears prudent, but it has been followed with highly variable stringency. The development of retinal degeneration has led to the hypothesis that this might be due to a shortage of essential fatty acids, such as linoleic and linolenic acids, sources of docosahexanoic acid (DHA), which is important in neural and retinal development. Monkeys deficient in DHA have had retinal degeneration [51], and there has been evidence of retinal dysfunction in premature infants that has been related to DHA [52]. DHA levels have been found to be low in patients with LCHAD deficiency [53]. For these reasons, DHA supplementation has been initiated in patients with LCHAD deficiency [26] and this approach has increased levels of DHA in the blood. In four patients studied, there was electrophysiological evidence of visual improvement. An 11-year-old boy with LCHAD peripheral neuropathy had improved nerve conduction data after 12 months of treatment with cod liver oil, which is high in DHA [54], however supplementation in other patients made no difference. Monitoring of change in the acylcarnitine profile has been reported to be useful in overall management, especially in MCT supplementation [33].

Treatment with creatine has been reported to be followed by a decrease in muscle pains and improved levels of CK in a single patient [55].

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42

Short-chain acyl CoA dehydrogenase (SCAD) deficiency

MAJOR PHENOTYPIC EXPRESSION

Predominantly neurological phenotype of hypotonia, myopathy, developmental delay, microcephaly, seizures, and failure to thrive [1].

INTRODUCTION

SCAD deficiency is an autosomal recessive short-chain fatty acid oxidation disorder. An estimated birth prevalence of 1:33,000–1:50,000 was reported, based on high C4-carnitine levels on newborn screening, and the presence of ACADS gene mutations on both alleles [2, 3]. SCAD is a member of the acyl-CoA dehydrogenase (ACAD) family of mitochondrial enzymes of the fatty acid ß-oxidation pathway [4]. SCAD catalyzes the dehydrogenation of the fatty acylCoA compounds of chain length f to 6 carbons (Figure 42.1) [5, 6], transferring electrons to electron transfer flavoprotein (ETF). SCAD is a tetrameric mitochondrial flavoenzyme, consisting of four subunits which are nuclear encoded and synthesized in the cytosol as precursor proteins. These are subsequently imported into the mitochondrial matrix to be proteolytically processed, folded and assembled into the biologically active SCAD homotetramer (168 Da) [7]. The SCAD enzyme functions in the mitochondria [4, 7]. Each SCAD monomer contains one molecule of its cofactor, flavin adenine dinucleotide (FAD) [8]. FAD binding to the SCAD enzyme is important for its catalytic activity, folding, assembly and/or stability [9–11]. The optimum substrate of the SCAD enzyme is butyrylCoA (C4-CoA); none of the other ACADs are active on this substrate *in vivo*. Deficiency of SCAD results in accumulation of butyrylCoA, which can be converted to butyrylcarnitine [12], butyrylglycine and carboxylated by propionylCoA carboxylase to ethylmalonic acid (EMA) [13]. The biochemical hallmark of SCAD deficiency is, therefore, raised levels of butyrylcarnitine which is evident on plasma acylcarnitine analysis, and EMA which is observed on measurement of urine organic acids. It is noteworthy that elevated EMA is not specific to primary SCAD deficiency, as it is also elevated in ethylmalonic encephalopathy [14, 15], respiratory chain defects [16–18] (Chapters 47–56) and multiple acyl-CoA dehydrogenase deficiency [19] (Chapter 45). EMA is, therefore, considered a nonspecific biochemical marker of SCAD enzyme dysfunction [20]. It does not correlate well with the severity of SCAD enzyme deficiency [1].

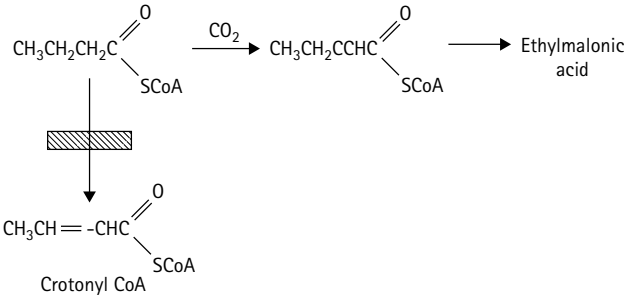

Figure 42.1 The SCAD reaction with butyryl CoA as a substrate. The conversion to ethylmalonylCoA is catalyzed by propionyl CoA carboxylase.

After the first patients with SCAD deficiency were reported [12, 21, 22], human SCAD cDNA was cloned by Naito *et al.* [8] based on its homology to the rat sequence. Subsequently, two pathogenic mutations were confirmed by Naito *et al.* at positions 136 and 319 of the coding region of the SCAD gene, which resulted in substitution of Arginine 22 and Arginine 83 with Tryptophan (Trp) and Cysteine

(Cys), respectively [23]. Subsequently, the SCAD encoding gene, *ACADS,* was localized to the terminal region of the long arm of chromosome 12, spanning approximately 13 kb, and consisting of 10 exons [24]. In the literature, approximately 38 different *ACADS* mutations have been described in patients with SCAD deficiency. Amongst those reported are c.136C>T, c.319C>T [23], c.1147C>T, c.274G>T,c.529C>T [25], c. 268G>A, c.575C>T, c.973C>T, c.1058C>T, c.1138C>T [20], c.332C>T, c.409C>T [26], c.417G>C, c.1095G>T [27], c. 1138C>T, c.1058C>T, c.989G>A, c.988C>T, c.1170C>G, c.136C>T [3], and c.256G>T, c.820G>A,c.826G>A,c.1108A>G [1]. These rare inactivating mutations, most of which are missense, lead to a complete deficiency of SCAD activity.

There are two common *ACADS* variants which have been identified in patients with SCAD deficiency, c.625G>A (p.Glycine (Gly) 209 (Serine) S) and c.511C>T (p.Arg171Trp). These common variations are overrepresented in homozygous or compound heterozygous form in up to 69 percent of patients with elevated levels of EMA (>18 mmol/mol creatinine), but are also found in 14 percent of the general population [20, 25, 29]. In the US, analysis of 694 newborn blood spots revealed an allele frequency of 22 percent for the c.625G>A variant and 3 percent for the c.511C>T [30]. These variants were detected in either homozygous or compound heterozygous form in 7 percent of the study population. In another study on newborn screening blood spots in the Netherlands, analysis of 1036 screening cards revealed 5.5 percent homozygosity and 31.3 percent heterozygosity for the c.625G>A variant [31].

The majority of patients with SCAD deficiency are homozygotes or compound heterozygotes for two of the common *ACADS* gene variants (c.625A/625A, c.511T/511T, c.625A/511T) or have a combination of these variants on one allele, with an inactivating mutation on the other allele [1, 3, 28, 32]. Corydon *et al.* [20] reported a study of 10 patients with ethlymalonic aciduria and deficiency of SCAD activity in fibroblasts. Sequence analysis revealed only one patient with a pathogenic mutation on both alleles. Five patients were doubly heterozygous for a pathogenic mutation on one allele, and common variation 625G>A in the other, while four other patients had either c.625G>A or c.511C>T on each allele. Waisbren *et al.* [32] described 14 patients with SCAD deficiency, of whom eight were identified from newborn screening, and six were patients identified on clinical presentation. All of the clinically identified children were homozygotes or compound heterozygotes for the common variants, except for one patient who also carried a 136C>T mutation. Pedersen *et al.* [1] reported the ACADS gene variation spectrum in 114 patients with SCAD deficiency identified on the basis of ethlymalonic aciduria, elevated butyrylcarnitine in plasma and/or fibroblasts, and decreased SCAD enzyme activity in fibroblasts or muscle. All but four patients were clinically symptomatic. The c.625G>A and the c.511C>T variations were present in 67 percent and 8 percent of the investigated alleles, respectively, compared to 21 percent and 8 percent in 100 alleles from Danish controls. Eleven of the 114 patients carried rare ACADs gene variations on both alleles, 39 were compound heterozygotes for a rare and a common variation, and 64 were homozygous or compound heterozygous for two common variations. Tein *et al.* [28] reported 10 patients of Ashkenazi Jewish ancestry with variable neuromuscular symptoms, three out of the 10 patients were homozygous for c.319C>T, the remaining seven had C.319C>T on one allele, and common variant c.625G>A on the other. The authors performed a population screening survey of c.319C>T in 105 individuals of Ashkenazi Jewish descent and found a carrier frequency of 1:15, and a 1:900 homozygote frequency, suggesting a likely founder effect. The incidence of SCAD deficiency, as well as the other diseases of fatty acid oxidation, is reportedly lower in Asian populations, in comparison to Caucasians [33]. The frequency of the c.625G>A variant in the Hispanic population (30%) is reported to be significantly higher than that of the African-American (9%) and Asian (13%) subpopulations [30].

The role of these common variants in the pathogenesis of SCAD deficiency remain poorly understood. The frequency of homozygosity for one of these variations in the general population suggest that they are not sufficient alone to cause SCAD disease. Alternatively, they are likely to confer susceptibility [20, 25, 34]. It has been suggested that these variants lead to disease in combination with genetic and/or environmental factors [20]. Apropos under specific conditions of stress such as elevated temperature, or in conjunction with an inactivating mutation or any other gene modifier, these variants could lead to reduced SCAD activity and result in SCAD disease [25].

The presence of SCAD variants and/or biochemical evidence of SCAD enzyme dysfunction in apparently unaffected individuals, as has been shown in family members of probands [1, 28], or asymptomatic patients identified on newborn screening, have raised questions of their clinical relevance [3, 35]. The benefits of an early diagnosis is unclear [36, 37]. The natural history remains poorly understood. There is insufficient evidence for optimal treatment [38]. The American College of Medical Genetics published an expert panel report which recommended the exclusion of SCAD deficiency from the core panel of fatty acid oxidation disorders screened. However, since SCAD deficiency is in a differential of the core diseases, it was advised that SCAD deficiency be retained as a secondary target [33]. Newborn screening for SCAD deficiency is performed in 35 of 51 states in the United States (National Newborn Screening Status Report NNSGRC 2008), as well as in Austria and Belgium [38]. In Great Britain, Denmark, and the Netherlands, SCAD deficiency is not included in the programs of newborn screening [38].

CLINICAL ABNORMALITIES

The reported age of onset is from the neonatal period [21] to adulthood [20]. In most cases, onset is before five years of age [1, 3, 28]. In the largest series to date of patients with

SCAD deficiency, Pedersen *et al.* [1] reported the age range of onset to be from 0 to 50 years of age, of whom 25 percent presented on the first day of life, 61 percent in the first year of life, and 4 percent after the age of 10 years. The initial reported patients with enzymatically-confirmed SCAD deficiency were of neonatal onset, one of which was fatal in the first week [21]. Since then, the clinical spectrum of SCAD deficiency has been extended to milder presentations [39]. The clinical course is unpredictable. Patients with SCAD deficiency have been reported who have had transient symptoms [3, 32], as well as patients who have improved to baseline over time [1, 3]. The spectrum of clinical features is difficult to correlate to the level of SCAD enzyme activity [3, 34], and there are no consistent genotype-clinical phenotype correlations [1, 3, 25]. There are also patients with SCAD deficiency who remain symptom free for many years after diagnosis, as reported in newborn screening follow-up studies [32, 41–43], or in family studies [28].

In contrast to the other ACAD deficiencies, which are more likely to present with hypoketotic hypoglycemia, hepatic or cardiac dysfunction, the clinical features of SCAD are predominantly neurological [1, 25, 28, 40]. Developmental delay is the commonest manifestation. Pedersen *et al.* [1] reported this in 69 percent of the 114 patients with SCAD deficiency. This was also described in another patient series [3, 28]. The other common symptoms are speech delay and hypotonia (Figure 42.2) [1, 28]. Other frequently reported features are seizures [1, 3], myopathy [1, 28], failure to thrive and feeding difficulties [1, 28], lethargy [28], and behavioral problems [3]. Some do have hypoglycemia [1, 3]. Less frequently seen are dysmorphic features, cardiomyopathy, intrauterine growth retardation or respiratory distress [1]. There has been one single reported case of abnormal cortical gyration and hypoplastic corpus callosum [44]. Acute metabolic episodes were not commonly reported [28]. Waisbren *et al.* [32] reported five of 14 patients with SCAD deficiency diagnosed on newborn screening, whose mothers had acute fatty liver of pregnancy with pre-eclampsia and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome. There had been one prior reported case [45].

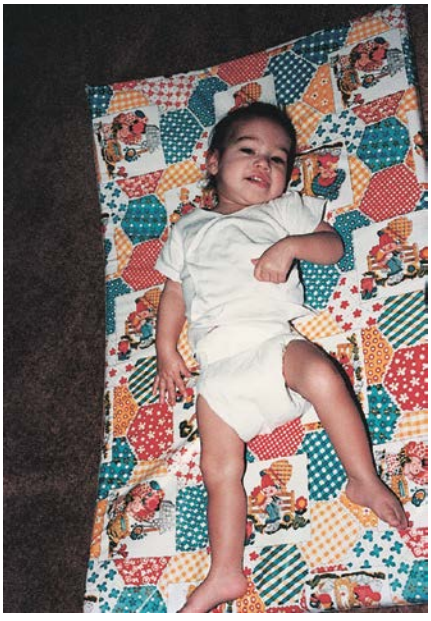

Figure 42.2 JG, an infant with SCAD deficiency. She was markedly hypotonic and had skeletal muscle weakness. Concentrations of free-carnitine in blood and muscle were low [1]. (Illustration was kindly provided by Dr. Susan Winter, Fresno, California.)

An initial biochemical evaluation of SCAD deficiency should include urinary organic acid profile, plasma acylcarnitine profile, and plasma carnitine [46]. Organic acid analysis characteristically reveals increased excretion of EMA. It is pertinent to note that though an elevated urinary EMA is characteristic, it is not diagnostic of SCAD deficiency. At times of relative stress, methylsuccinic acid may be excreted in the urine [21]. Butyrylglycine and butyrylcarnitine may also be found in urine [47–49]. The urine can be normal at times of relative health. This underlines the intermittent nature of the excretion of EMA, which appears to be dependent upon the degree of intercurrent metabolic stress [1]. Butyrylcarnitine should be distinguished from isobutyrylcarnitine, which may be found in normal individuals [50], ethylmalonic encephalopathy [14], and in patients with a defect in branched-chain acyl CoA oxidation, isobutyryl CoA dehydrogenase deficiency [51]. These distinctions may be made by organic acid analysis, enzyme assay, mutational analysis or study of the accumulation of labeled acylcarnitines *in vitro*.

In newborn screening programs, SCAD deficiency is detected by acylcarnitine measurement using tandem mass spectrometry (MS/MS) on plasma or blood spots. The key compound is C_4, butyrylcarnitine (Figure 42.3), but C_5 may also be elevated. The diagnosis may subsequently be confirmed by performing enzymatic assays in skin fibroblasts. ^{14}C-butyrate uptake studies have previously served as a screening test for this disorder [53]. Determination of SCAD enzyme activity requires butyryl CoA as a substrate [52], and incubation with anti-medium-chain acyl CoA dehydrogenase (MCAD) antibody, in view of the overlapping activity of MCAD toward C4-C6 acylCoAs [21, 53, 54]. However, enzyme analysis in fibroblasts or lymphocytes has demonstrated high residual SCAD activity in some patients with SCAD deficiency [27, 55], or has given inconsistent results [20, 25]. Measurement in skeletal muscle is reported to be more reliable than skin fibroblasts [55]. Confirmation of the diagnosis of SCAD deficiency is typically by molecular analysis of the *ACADS* gene, with full sequencing if required.

GENETICS AND PATHOGENESIS

SCAD deficiency continues to be a major diagnostic management dilemma because of the marked genetic heterogeneity and clinical unpredictability of the *ACADS* gene spectrum, and the lack of correlation between

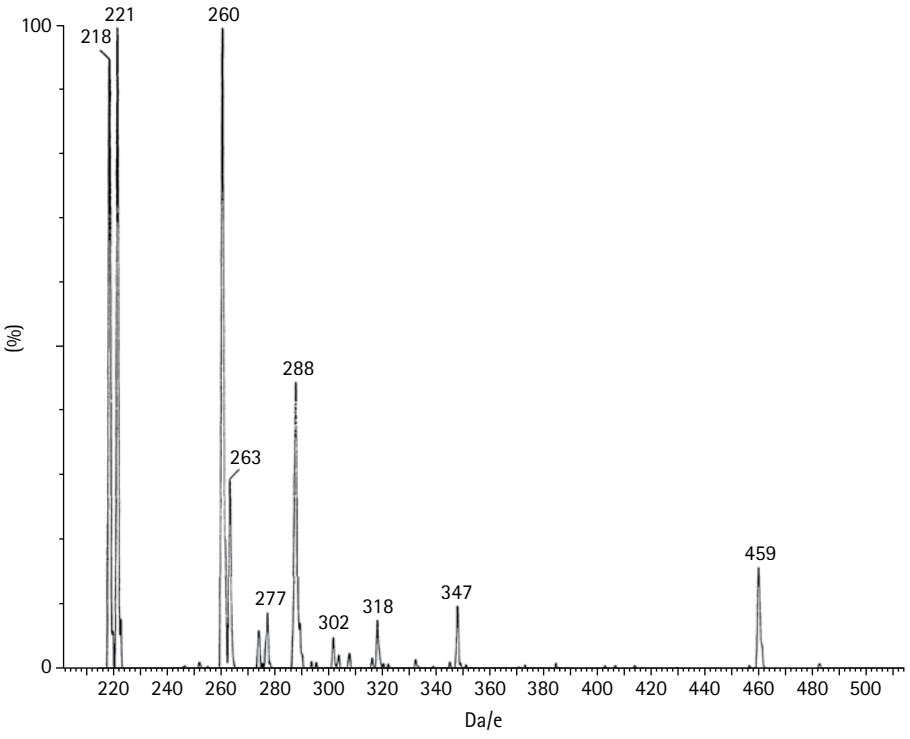

Figure 42.3 Acylcarnitine tandem MS profile of a 3-year-old patient with SCAD deficiency. He presented with myopathy and hepatomegaly. He had episodes of hypoglycemia and lactic acidosis. He died of cardiomyopathy. The key compounds are: 288 C4-carnitine and 302 C5-carnitine.

genotypes, clinical phenotypes, and pathophysiology. Symptoms of SCAD deficiency are primarily neurologic, and the reasons for the neurotoxicity are still not understood. Gregersen *et al.* [56] highlighted the expanse of the *ACADS* gene spectrum, compared to those of the other ACADS such as MCAD and VLCAD. In addition to the extensive spectrum of inactivating variations, the two common variants c625G>A and c.511 C>T are found in up to 14 percent of the general population in homozygous or compound heterozygous form [25, 30]. In 293 symptomatic patients and 45 screened newborns, 58 *ACADS* variations were found, 50 of which were missense mutations, and one was an in-frame deletion. In the symptomatic patient group, 10 percent carried two rare inactivating mutations, 34 percent carried one inactivating variant on one allele, and a common variant on the other allele, and 56 percent were either homozygous or compound heterozygotes for a common variant. On the other hand, the proportion of patients in the screened newborn group who carried two inactivating variations was much higher at 65 percent. Thirteen percent carried one inactivating, and one common variant, and 22 percent were homozygous or compound heterozygous for a common variant. It is not understood why a significant number of the screened newborns carrying rare inactivating mutations, or their family members carrying the same rare variants, would remain asymptomatic. Conversely, it is not clear why a higher proportion (56%) of symptomatic patients are either homozygous or compound heterozygotes for the common variations, most often the c.625G>A variant [1, 20, 25, 28].

Patients homozygous for inactivating mutations show more severe biochemical abnormalities with very low levels of SCAD activity [1, 28, 57, 58], compared to those with one inactivating variant and one common variant [55]. Patients who are homozygous or compound heterozygous for the common variants have either normal or variably decreased levels of SCAD activity [25]. This may explain why only 22 percent of screened newborns have the common variants, as some individuals who are homozygous or compound heterozygous for a common variant may not have butyrylcarnitine levels above the newborn screening cut-off level and remain unidentified [32]. Measurement of common variant proteins p.Gly209Ser encoded by c.625G>A, and p.Arg147Trp, encoded by c.511C>T in *E. coli* at 37 degrees showed 86 percent and 69 percent activity of the mean wild-type SCAD value [20]. Expression of the p.Gly209Ser variant protein in Cos-7 cells revealed corresponding lower SCAD activity levels at higher temperature, reducing from 45 percent to 13 percent, with temperature increase from 37 to 41 degrees respectively. The p.Arg147Trp SCAD protein in Cos-7 cells also showed decreasing activity, but from higher than normal levels at 37 degrees, reducing to 58 percent at 41 degrees. Catalytic function of the purified p.Gly209Ser variant protein in *E. coli* has been shown to be impaired compared to wild-type SCAD, whereas the p.Arg147Trp was similar to wild-type [59]. Subsequently, *in vitro* import studies of inactivating variant proteins, including the common variant SCAD proteins, in isolated mitochondria from SCAD deficient mice demonstrated an increased tendency to protein misfolding and aggregation [1, 57]. The mechanism whereby misfolding and aggregation leads to cellular toxicity has been thought to relate to the accumulation of toxic aggregates, or toxicity of soluble oligomeric species [60]. SCAD variant protein misfolding

was also shown to be temperature dependent [1, 57]. This further underlines the relevance of environmental and/or genetic factors in the pathophysiology of SCAD disease. This is particularly relevant in the understanding of the pathophysiology of the susceptibility variants c.625G>A and c.511C>T. Pedersen *et al.* [61] investigated fibroblast cell lines from 10 symptomatic patients with c.625G>A homozygosity, compared to four asymptomatic individuals with the same c.625G>A homozygosity and control fibroblasts (n = 24) which were confirmed to have normal SCAD function without c.625G>A homozygosity. The symptomatic patient lines were shown to have significantly reduced levels of SCAD activity, and reduced expression of SCAD mRNA. Superoxide Dismutase 2 (SOD2), a major intramitochondrial antioxidant enzyme, was reduced on quantitative proteomic assay by nano-LC-MS/MS and confirmed by Western blot. There was also increased sensitivity to menadione-induced oxidative stress, compared to controls and healthy c.625G>A homozygote cells. This is consistent with increased sensitivity to oxidative stress in the c.625G>A patient cells. Conversely, the fibroblasts from healthy individuals with C.625G>A homozygosity revealed comparable levels of SCAD protein expression and activity to the control group, and instead had higher SOD2 expression on proteomic analysis and Western blot, in conjunction with increased resistance to menadione-induced oxidative stress. This suggested increased resistance to oxidative stress in cells from c.625G>A healthy individuals. Sequence analysis of all of the exons and a large part of the promoter in the SOD2 gene revealed identical haplotyes in all groups. It is speculated that SOD2 gene dysregulation could therefore be a factor. Antioxidant dysfunction and increased susceptibility to oxidative stress may therefore contribute to the pathophysiology of SCADD [61]. Furthermore, Schmidt *et al.* [62] reported that the c.319C>T SCAD protein p.Arg83Cys was unable to attain the normal soluble conformation when expressed in an astrocytic cell line, and therefore prone to accumulate in the insoluble fraction. Concomitantly, fission of the mitochondrial network was evident, which was likely to be due to oxidative stress. This further supports the hypothesis of protein misfolding, oxidative stress and mitochondrial dysfunction as contributory pathogenetic mechanisms.

Physiologic testing, including the response to fasting and loading with fat, was explored in 15 patients in Holland [63]. All had been ascertained on the basis of clinical manifestations, four because of hypoglycemia. The diagnosis of SCAD deficiency was made on the basis of an increase in EMA in the urine or C_4-carnitine in plasma. All were genotyped, and they fell into three groups: mut/mut, mut/var, and var/var, with the common polymorphic mutations c.511C>T and c.625G>A considered variants. Hypoglycemia developed in three patients during fasting. One of these was fasted two years later without hypoglycemia, which the authors interpreted as consistent with idiopathic ketotic hyperglycemia. Hypoketosis in response to fasting was seen only in the patient who initially had hypoglycemia and later was normal. The later test was not hypoketotic. Excretion of ethylmalonic acid increased in all groups with fasting, and the increase was greater in the mut/mut group.

TREATMENT

The efficacy of treatment is unclear. Patients have been treated with carnitine and restriction of dietary fat. Prolonged fasting should be avoided. Van Maldegem *et al.* [64] reported a lack of clinical improvement to high-dose riboflavin.

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43

Short-chain 3-hydroxyacylCoA dehydrogenase (SCHAD) deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, recurrent myoglobinuria, encephalopathy, and cardiomyopathy; or hyperketotic hypoglycemia, failure to thrive, and hypotonia; elevated creatine kinase; dicarboxylic aciduria; and defective activity of HADH in muscle fibroblasts and leukocytes. Some patients with familial hyperinsulinemia hypoglycemia (HHF_4) have mutations in the *HADH* gene.

INTRODUCTION

Deficiency of hydroxyacylCoA dehydrogenase (HADH) (EC 1.1.1.35) was first described by Tein and colleagues [1] in a 16-year-old girl with recurrent myoglobinuria and hypoketotic hypoglycemia in whom HADH activity was markedly diminished in muscle, but normal in fibroblasts. In contrast, we and others have seen patients in whom enzyme activity was very low in cultured fibroblasts and freshly isolated leukocytes [2]. These patients have had what appeared to be ketotic hypoglycemia.

The enzyme, 3-hydroxyacyl CoA dehydrogenase, is a homodimer with 302 amino acids in each subunit [3–5] with activity against 3-hydroxyacylCoA esters of C4 to C16 length, but with greatest activity against C10 and less activity as the chain length increases (Figure 43.1). The cDNA for the gene has been cloned and sequenced [6, 7] and mapped to chromosome 4q22-26 [7]. It contains eight exons and spans 49 kb. The enzyme is synthesized with a leader peptide, which is removed after import into the mitochondria. Mutations have been identified [8].

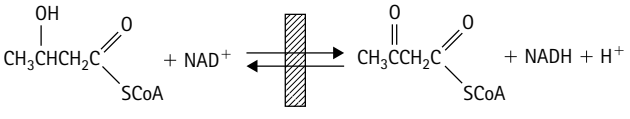

Figure 43.1 The 3-hydroxyacylCoA dehydrogenase reaction. Substrates 3-hydroxybutyryl CoA and the ketoacid product. The enzyme catalyzes conversion of C_4 to C_{16} esters.

CLINICAL ABNORMALITIES

The initial patient [1] with deficiency of HADH had episodic myoglobinuria and hypoketotic hypoglycemia, as expected for a disorder of fatty acid oxidation. There was also evidence of encephalopathy and hypertrophic dilated cardiomyopathy.

Our patient [2] had a neonatal presentation of difficulty with feeding, failure to thrive, and hypotonia. An elevated creatine phosphokinase (CPK) of 2000 U/L led to a muscle biopsy, which appeared normal. The CK was recorded as high as 5721 U/L. In response to fasting, she developed hypoglycemia of 38 mg/dL at 23 hours, but the concentration of 3-hydroxybutyrate in the blood was 2120 mmol/L, which was a brisk ketogenic response.

Sudden infant death has also been reported [9, 10]. One such infant had brick red urine, indicative of myoglobinuria. Autopsy revealed a fatty liver. Fulminant hepatic failure has also been observed, treated by liver transplantation [10].

Other patients have presented with a picture of hyperketotic hypoglycemia [10]. There may be vomiting, dehydration, or lethargy; or onset may be with seizures. One patient had hyponatremia [10]. The liver may be enlarged.

Another presentation is with hyperinsulinemic hypoglycemia [11, 12]. In one Pakistani family in which parents were doubly heterozygous, hyperinsulinism required treatment with diazoxide [8]. Of four affected children, two died. One had impaired mental development and one had developed normally. An infant in another family had hyperinsulinism that was readily controlled with diazoxide and hydrochlorthiazide [11].

Laboratory evaluation has revealed hypoketosis in some patients [1, 10]. Analysis of the free fatty acids of the plasma or organic acid analysis of the urine may reveal medium-chain 3-hydroxy fatty acids, even when the patient is metabolically well [12, 13]. However, this pattern may also be seen in infants receiving medium-chain triglycerides.

The concentration of free carnitine in the plasma may be normal or low, and there may be increased quantities of carnitine esters in the urine. Medium-chain dicarboxylic aciduria is characteristic, but the levels are not high, and at times they may be normal. Adipic, suberic, and sebacic acids are found, as well as 3-hydroxydicarboxylic acids. In our patient, challenge with a load of medium-chain triglyceride led to increased excretion of dicarboxylic acids and 3-hydroxydicarboxylic acids. She always excreted elevated amounts of trans-cinnamoyl glycine.

The acylcarnitine profile reveals C_4-OH carnitine which could indicate the 3-hydroxybutyrylcarnitine of HADH disease, but this would not be different in the presence of 3-hydroxyisobutyrylCoA deacylase deficiency or D-3-hydroxybutyrylcarnitine in a ketone body utilization defect. In one patient [9], elevated C16 and C18 acylcarnitines were found, and no hydroxyacylcarnitines.

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion. Consanguinity has been observed and heterozygosity has been demonstrated by enzyme assay of the liver [9] and by mutational analysis [12].

The enzyme HADH is a soluble mitochondrial matrix enzyme with two identical subunits [3]. The gene encodes a 34-kDa precursor protein that, with processing, yields a mature 31-kDa subunit. Its substrate specificity is considerably broader than the name would suggest. It is most highly active against hydroxybutyryl CoA, but it is active up to C16. Deficiency of enzyme activity has been demonstrated in muscle [1], liver [9], and fibroblasts [12], as well as in mitochondria isolated from fibroblasts [11]. In some families, the defective enzyme was not demonstrable in fibroblasts, but was found in muscle [1] or liver [9].

The rat and human cDNAs have been sequenced [6, 7]. The human gene encodes a protein of 314 amino acids and is expressed in the liver, kidney, heart, and muscle. The gene contains eight exons. Compound heterozygosity for two mutations (A118G p.A28T and C171 A p.D45E) has been observed in a patient with hepatic failure. In an Indian patient with hyperinsulinemic hypoglycemia, heterozygosity was found for C773T resulting in p.P258L. In a consanguineous Pakistani family, a deletion was found that removed the acceptor splice site adjacent to exon 5 and led to deletion of exon 5 in the mRNA [14].

A novel homozygous mutation was found [15] in a family with severe neonatal hypoglycemia and hyperinsulinemia. Activity of SCHAD in fibroblasts was greatly reduced, and levels of 3-hydroxybutyrylcarnitine in plasma were increased. Levels of 3-hydroxyglutaric acid were increased. This SCHAD deficiency can result in persistent hyperinsulinemic hypoglycemia of infancy (PHHI).

TREATMENT

Treatment with carnitine and a diet low in fat appears to be prudent. The avoidance of fasting is important and supplemental corn starch may be useful. Treatment with glucagon and diazoxide has been employed in the severe hyperinsulinemic hypoglycemic patients [15]. A link between fatty acid oxidation and the dysregulation of acid oxidation and the dysregulation of the secretion of insulin has been clarified by studies in *hadh* -/- mice [16]. These mice had increased islet sensitivity to leucine. SCHAD deficiency appears to cause hyperinsulinemia by activation of glutamic dehydrogenase (GDH) via loss of the normal inhibitory regulation of GDH by SCHAD.

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44

Short/branched-chain acyl-CoA dehydrogenase (2-methylbutyrylCoA dehydrogenase) deficiency

MAJOR PHENOTYPIC EXPRESSION

Episodic lethargy, hypoglycemia, and acidosis; hypotonia; impaired mental development; possibly asymptomatic; 2-methylbutyrylglycinuria; 2-methylbutyrylcarnitinemia; and short/branched-chain acyl-CoA dehydrogenase deficiency.

INTRODUCTION

Short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency may be an inborn error of metabolism, which does not manifest clinically unless the patient undergoes some level of metabolic stress. Of two affected siblings in the initial report of Gibson and colleagues in 2000 [1], the first manifested neurologic abnormalities following a probable ischemic/hypoxic event at three days of age. His sister, identified prenatally, had been completely asymptomatic by the time of follow-up report [2], and a number of other patients have been asymptomatic, particularly those identified by neonatal screening [2–5].

The key to the metabolic abnormality was the excretion of 2-methylbutyrylglycine in the urine and an elevated level of 2-methylbutyrylcarnitine in the blood. The activity of 2-methybutyryl CoA dehydrogenase (Figure 44.1) in fibroblasts was found to be deficient [3] and Western blot revealed absence of the enzyme in one family [1]. The cDNA for the *SBCAD* gene was isolated by Rozen *et al.* [6] and mapped to chromosome 10q25-q24 [7]. At least three mutations have been identified [1–3].

CLINICAL MANIFESTATIONS

The initial patient [1] was first admitted at three days of life with a life-threatening episode characterized by hypoglycemia, dehydration, lethargy, and hypothermia. Acidosis was mild and there was no massive ketosis. Magnetic resonance imaging (MRI) revealed increased signal in the lentiform nuclei, and the electroencephalogram (EEG) was abnormal. By 12 months of age, he was behind in visual, motor, and cognitive skills and carried a diagnosis of athetoid cerebral palsy. 2-Methylbutyrylcarnitine was found in the blood and 2-methylbutyrylglycine was found in the urine. In another family [2, 3], the patient was a three-year-old product of a consanguineous mating who had hypotonia and impaired motor development. MRI was normal. He had 2-methylbutyrylglycinuria, but a normal acylcarnitine profile. His asymptomatic mother also excreted 2-methylbutyrylglycine. Among ethnic Hmongs, eight patients have been found on expanded newborn screening to have 2-methylbutyrylglycinuria and a distinct mutation [4]. Except for mild muscular hypotonia observed at six months of age, all were asymptomatic. Of two other consanguineous patients, one had attention-deficient disorder, and one convulsive disease and developmental delay. It appears that patients with this disorder may be asymptomatic, but neurologic disease may be a feature.

GENETICS AND PATHOGENESIS

SBCAD deficiency is coded for by an autosomal recessive gene on chromosome 10 [6]. It was localized to 10q25-q26 by fluorescence *in situ* hybridization.

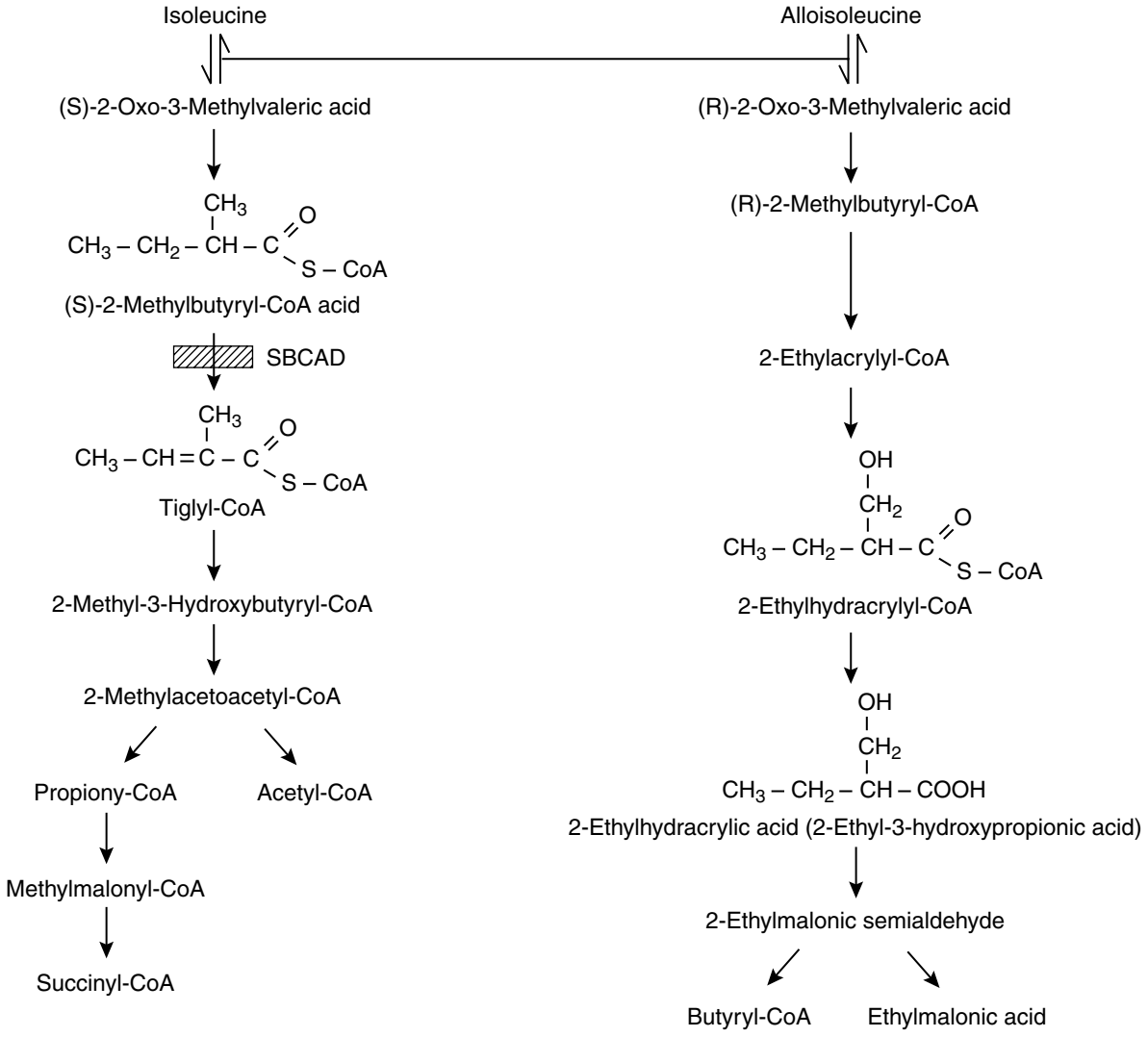

Figure 44.1 Metabolic pathways relevant to SBCAD deficiency. Interelations of the S and R pathways of isoleucine catabolism are shown.

The gene contains 11 exons [7]; its open reading frame of 1.3 kb encodes a precursor protein of 431 amino acids, which is processed to a mature protein of 399 amino acids. The SBCAD protein is imported into the mitochondria and forms a tetramer. The cDNA has considerable sequence homology with other acyl-CoA dehydrogenases (ACAD). Greatest homology is to short chain acyl-CoA dehydrogenase (SCAD) (Chapter 42) and ACAD-8 [8] which is an isobutyrylCoA dehydrogenase. The activity of SBCAD is greatest toward 2-methylbutyrylCoA, but it reacts with other 2-methylbranched chain substrates and with short chain acyl-CoA compounds, including butyryl-CoA. It has little or no activity against isobutyrylCoA.

Mutational analysis in the first patient revealed a C778T substitution in the coding region which led to the substitution of a phenylalanine for leucine at amino acid 22 [1]. Mutational analysis revealed homozygosity for a G1228A transition in the second patient and his mother [3]. This led to skipping of exon 10 and a 100-bp deletion. The patient was also heterozygous for the common A625 variant SCAD allele. Mutational analysis in the Hmong patients yielded a homozygous A1165G mutation, which also led to skipping of exon 10. Four additional mutations identified [9] were C443T, A848G, T1102C, and G9085C. These mutations led to defective activity of the SBCAD enzyme.

In the first patient [1], the conversion of ^{14}C-isoleucine to $^{14}CO_2$ in intact fibroblasts was impaired. Incubation of ^{13}C-isoleucine with L-carnitine in intact cultured fibroblasts led to accumulation of isotope in C5-acylcarnitine. Western blot analysis revealed absence of the SBCAD protein. In the second patient [3], the activity of 2-methylbutyrylCoA dehydrogenase in fibroblasts was 10 percent of control. Defective activity was also demonstrated by expressing the abnormal gene products in *E. coli* and COS cells [1–3]. In the Hmong patients, there was also no cross-reactive material (CRM) [4]. Prenatal diagnosis of an affected fetus has been accomplished [1].

Defective activity of SBCAD enzyme leads to accumulation of 2-methylbutyryl-CoA and its conjugation products 2-methylbutyrylglycine and 2-methylbutyryl (C5) carnitine. Tandem mass spectrometry (MS/MS) has been invaluable for the identification of this disorder of isoleucine metabolism. Many of those reported have been found through programs of expanded newborn screening. Elevated C5 acylcarnitine may be documented by analysis of the plasma, as well as of dried blood on filter paper. Plasma concentrations reported have varied from 0.7 to 3.4 μmol/L; controls were <0.6 [4]. In blood spots, levels ranging from 0.5 to 2.5 μmol/L have been observed; reference <0.46 [5]. 2-Methylbutyrylglycinuria is identified by organic acid analysis of the urine. Levels reported have ranged from 3 to 73 mmol/mol creatinine [1–5, 9]. Normal levels are generally less than 2 mmol/mol creatinine. It is clear from the range observed that some patients display a peak that is so small it could be missed on organic analysis. Acylglycines are considered to be recognized with inadequate sensitivity by gas chromatography-mass spectrometry (GCMS) because of variable extraction, chromatographic instability, or failure of spectrum recognition [9]. This has led to methods of stable isotope dilution, selected ion monitoring GCMS [10] or MS/MS [11]. Patients with this disease have also been found to excrete 2-ethylhydracrylic (2-ethyl-3-hydroxypropionic) acid (see Figure 44.1) [9] and this may serve as another recognition marker for organic acid analysis. The amounts of this compound usually exceed that of 2-methylbutyrylglycine. Quantification has not been perfect, for there is no commercial standard for 2-ethylhydracrylic acid, but comparison arbitrary units ranged from 8 to 152 in four patients (controls <5) [9]. Chiral determination of 2-methylbutyric acid indicated that 40–46 percent was in the form of the R isomer in patients and in controls. There are two pathways of isoleucine catabolism (see Figure 44.1). Isoleucine itself is predominately in S chiral form. It is transaminated to its keto acid, 2-oxo-3-methylvaleric acid, which is subsequently oxidized via the S pathway to (S)-2-methylbutyrylCoA where the next step is catalyzed by the SBCAD enzyme [5]. Keto-enol tautomeric racemization following or enamine tautomerization during, transamination is the source of alloisoleucine (Chapter 19). 2-Ethylhydacrylic acid is on the R pathway. Its occurrence in SBCAD deficiency indicates that this enzyme does not catalyze the conversion of R-2 methylbutyryl-CoA to 2-methylacrylylCoA.

Ethylhydracrylic acid excretion in increased quantity may also be observed in ketosis [12], in 3-oxothiolase deficiency [13], in 2-methyl-3-hydroxybutyrylCoA dehydrogenase deficiency [14], in propionic acidemia [15], and methylmalonic acidemia, all defects in steps of the S pathway. It may also be found in ethylmalonic encephalopathy, hydroxyisobutyric aciduria, and in Barth syndrome.

Prenatal diagnosis was accomplished [1] by analysis of 2-methylbutyrylglycine in amniotic fluid (0.27 μm/L, normal <0.03) and of C5-acylcarnitine (1.93 μm/L; normal 0.37 ± 0.18) [14].

TREATMENT

Patients have been treated with carnitine in doses of 50–100 mg/kg per day and diets restricted in protein or isoleucine [2, 4], but poor compliance and discontinuation have occurred without obvious clinical consequences [4]. A protein intake of 1–4 g/kg along with carnitine of 71 mg/kg led to a normal excretion of 2-methylbutyrylglycine [1].

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45

Multiple acyl CoA dehydrogenase deficiency/glutaric aciduria type II ethylmalonic-adipic aciduria

MAJOR PHENOTYPIC EXPRESSION

Overwhelming neonatal illness with metabolic acidosis, acrid odor, hypoketotic hypoglycemia, and hyperammonemia; dysmorphic features; polycystic kidneys; massive urinary excretion of lactic and glutaric acids, and increased concentrations of many other organic acids, including ethylmalonic acid, butyric acid, methylbutyric acid, isobutyric and isovaleric acids, and deficiency of electron transfer flavoprotein (ETF) or its dehydrogenase (ETF-QO). Later onset, milder variants referred to as 'ethylmalonic-adipic aciduria', may first present in the neonatal period or adulthood with episodic illness characterized by vomiting, hypoglycemia, and/or lipid storage myopathy.

INTRODUCTION

Glutaric aciduria type II was first reported in 1976 by Przyrembel *et al.* [1] in an infant with severe hypoglycemia and profound metabolic acidosis without ketosis. Patients with this form of the disorder have overwhelming illness in the neonatal period that has been uniformly fatal. The name was employed to distinguish the disease from the glutaric aciduria due to defective activity of glutaryl-CoA dehydrogenase (Chapter 9) that had been reported one year earlier by Goodman and colleagues [2]. Organic acid analysis revealed the accumulation of a wide variety of organic acids, including lactic, isovaleric, and ethylmalonic acids, as well as glutaric acid. There is generalized defect in the activity of at least 9 acyl CoA dehydrogenases [3]. Thus, the term "multiple acyl CoA dehydrogenase deficiency" (MADD) is more descriptive, it has variously been abbreviated MAD deficiency and MADD; it has also been divided into severe (MAD:S) and mild (MAD:M) forms [4], but there is sufficient heterogeneity of clinical expression.

The fundamental molecular defect is in the mitochondrial transport of electrons from the acylCoAs to ubiquinone (CoQ10) of the main electron transport chain [5–7]. The transfer of electrons from the 2,3 positions of a number of important energy-providing substrates requires the concerted activities of the electron transfer flavoprotein (ETF), a mitochondrial matrix protein, and the electron transfer flavoprotein: Ubiquinone oxidoreductase (ETF-QO), which is an inner mitochondrial membrane protein that transfers electron to coenzyme Q in the respiratory chain. The defect may be in any of three proteins, the alpha or beta subunits of ETF or its dehydrogenase, ETF-QO (EC 1.5.5.1). Both are flavoproteins. Another designation has been IIA and IIB for defects in the α and β proteins and IIC for ETF-QO defects.

The mitochondrial oxidations of glutaryl-CoA and other intermediates in branched-chain amino acid metabolism, and the β-oxidation of fatty acids (see Figure 45.1) are catalyzed by mitochondrial flavinadenine dinucleotide (FAD)-dependent enzymes [8–11]. Each of the dehydrogenase enzymes of fatty acid oxidation, and the amino acid catabolic enzymes catalyze the dehydrogenation of saturated acylCoA compounds to form the 2,3-unsaturated or enoylCoA thioesters (Figure 45.2). Both sarcosine and dimethylglycine are catabolized by specific N-methyldehydrogenases containing covalently bound FAD and dissociable folic acid cofactors [12–14],

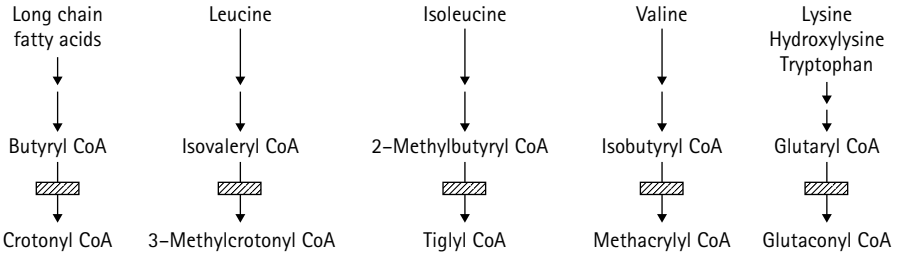

Figure 45.1 Multiple acyl CoA dehydrogenase deficiency (MADD) involves the dehydrogenation of many CoA intermediates in fatty acid and amino acid catabolism.

and thus these two compounds may also accumulate in this disease. Each dehydrogenase enzyme contains a molecule of FAD.

ETF is a mitochondrial matrix heterodimer containing an AMP molecule and a single noncovalently bound FAD which accepts hydrogens from all of the acylCoA dehydrogenases [15–19]. ETF has a 30-kD α- and a 28-kD β-subunit. ETF-QO is a 64-kD monomer iron-sulfur-containing (^{4}Fe-^{4}S) flavoprotein (previously referred to as Fe-S flavoprotein) that accepts electrons from reduced ETF and transmits them to coenzyme Q and the cytochrome chain (see Figure 45.2) [7, 20–22].

The cDNAs for the α- and β-subunits of ETF [23, 24] and ETF-QO [25] have been cloned and sequenced. α-ETF and ETF-QO have N-terminal mitochondrial import sequences, but the β-subunit cDNA does not encode a leader peptide, and so does not undergo such processing. The gene for α-ETF has been localized to chromosome 15 at q24.2-24.3 [26], that of β-ETF on chromosome 19 [24, 27] at q13.41, and that for ETF-QO on chromosome 4 [28] at q32.1. Mutations have been identified in α-ETF [30, 31], the most common of which appears to be a change at codon 266 from threonine to methionine resulting in neonatal disease, as well as in the β-ETF [29]. In ETF-QO, a number of apparently rare mutations have been identified which lead to an absence of enzyme activity and immunoreactive proteins [28]. Homozygosity for two null mutations of α-ETF, β-ETF, or ETFDH is associated with neonatal onset with anomalies (type I), whereas small amounts of residual activity prevent the development of embryonic anomalies (type II). ETFDH mutations are the major causes of riboflavin-responsive MADD [29].

Preliminary data from expanded newborn screening programs have estimated a prevalence of MADD of 1 in 750,000 to 2,000,000 newborns [32]. There appears to be a much higher incidence in the Turkish population, > 1:20,000 [33].

CLINICAL ABNORMALITIES

The infant with classic MADD presents with life-threatening illness in the first days of life. The clinical picture is reminiscent of those of the typical organic acidemias, propionic acidemia (Chapter 2), methylmalonic acidemia (Chapter 3), and isovaleric acidemia (Chapter 8), but the severity of illness in this disease is so great that all three of the patients we have studied died after less than 90 hours of life [34, 35], and most of those reported have died within the first week [1, 36–41].

These infants [1, 34] develop tachypnea or dyspnea within a few hours of birth. They are found to have profound metabolic acidosis and impressive hypoglycemia. In spite of intravenous glucose and $NaHCO_3$, the concentration of glucose in the blood may decrease as does the pH, and cardiac arrest soon follows, and despite resuscitation and artificial ventilation, the course is inexorable.

The first patient was described as having a "very disagreeable sweaty-feet odor" [1]. We described our first patient [34] as having a peculiar, acrid odor. This is the consequence of an excess of a number of short-chain, volatile organic acids. A number of these patients have been described as pale [1, 34, 35] and one had macrocytic anemia and a hemoglobin concentration of 9.1 g/dL. Many have had convulsions consistent with the degree of depression of the blood glucose. Hyperammonemia was a consistent feature in our patients [34, 35]. Fatty infiltration of the liver is found postmortem.

A number of the neonatal onset patients have had prominent dysmorphic features (Figures 45.3–45.8) [34, 35, 42]. They include a high forehead, depressed nasal bridge, and a short anteverted nose. The ears may be low-set, malrotated, and abnormally formed (Figures 45.3, 45.5, and 45.6). Muscular defects of the abdominal wall have occurred, as well as genital defects, such as hypospadias and chordee. Some have had macrocephaly [43]. Minor

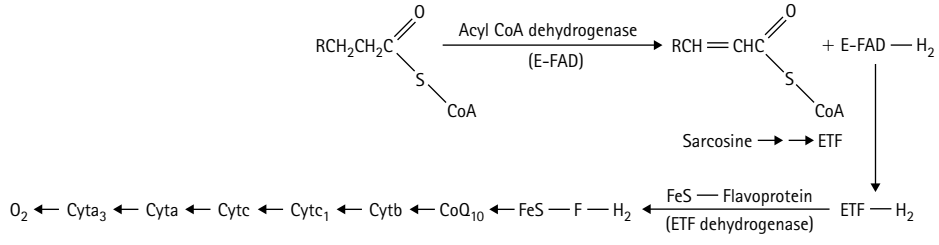

Figure 45.2 The roles of electron transfer flavoprotein (ETF) and ETF-QO oxidation and the passage of electrons along the electron transport chain.

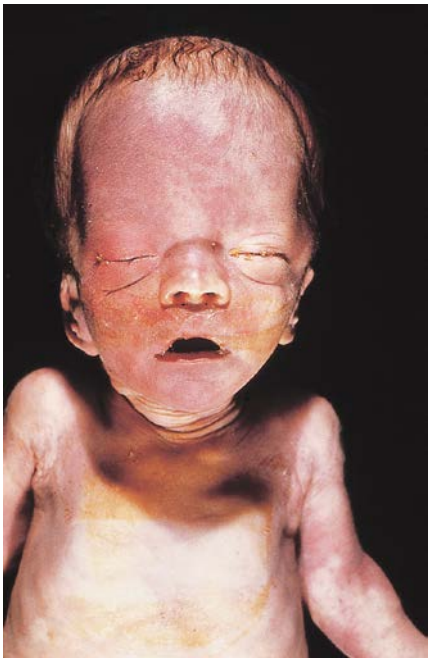

Figure 45.3 Postmortem pictures of Baby M [34] who died of glutaric aciduria type II on the first day of life. He had a low, incompletely rotated, low-s ears with a reduced anthelix, semilunar folds below the eyes and three umbilical vessels. Autopsy revealed large polycystic kidneys and an interventricular septal defect.

anomalies include horizontal palmar creases and rocker-bottom feet (Figure 45.4). One of our patients also had an interventricular septal defect and three umbilical vessels. Some patients [34] have been described as premature or small for gestational age.

A major malformation in these infants is the occurrence of polycystic kidneys. The kidneys may be huge and readily palpable. Abnormally small prenatal production of urine may be the cause of the semilunar folds below the eyes, as in the Potter syndrome (Figure 45.3), and typical Potter syndrome, including pulmonary hypoplasia has been observed [37]. Polycystic kidneys may be present in

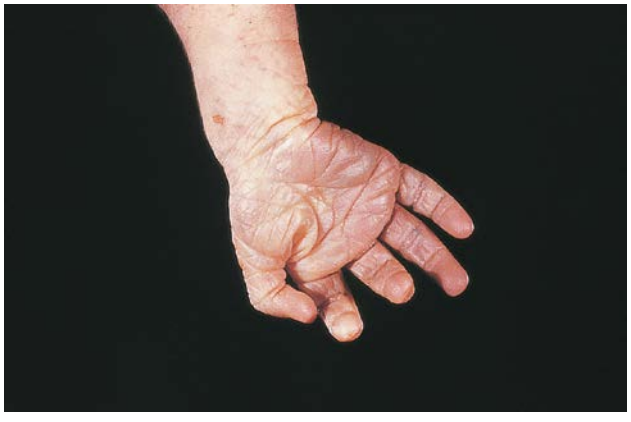

Figure 45.4 The hand was short and broad, and had a single horizontal crease.

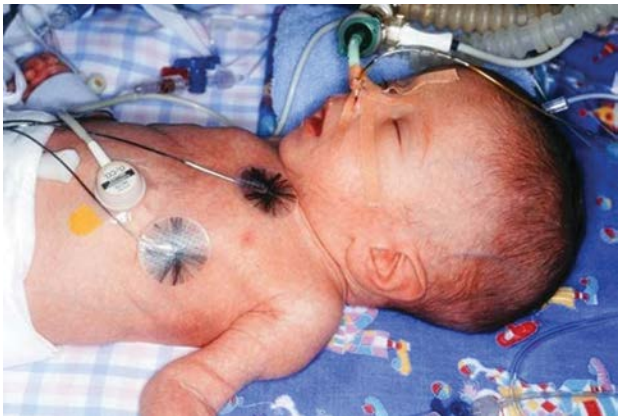

Figure 45.5 Baby K died of intractable acidosis at 3 days of life. He had a high forehead, depressed nasal bridge, short nose with anteverted nares, a long philtrum and micrognathia. The ears were low-set.

infants without dysmorphic features and may be found first at autopsy [38, 42]. Ultrastructural changes have been described in the glomerular basement membrane [43]. Other pathologic abnormalities include cerebral gliosis and heterotopias giving a warty dysplastic appearance to the cortex [37]. Electron dense membrane limited bodies have been reported in the brain and kidneys [41]. Hepatic periportal necrosis has been reported [43], and more commonly hepatic microvesicular lipid. Pancreatic ducts may be hypoplastic [37]. Recurrent pancreatitis may occur later in milder cases, and this possibility must be investigated when abdominal symptoms of unknown origin occur [44].

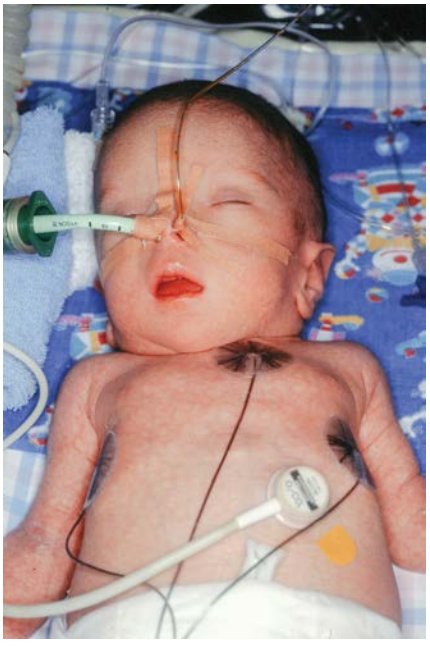

Figure 45.6 Baby K died of intractable acidosis at 3 days of life. He had a high forehead, depressed nasal bridge, short nose with anteverted nares, a long philtrum and micrognathia. The ears were low-set.

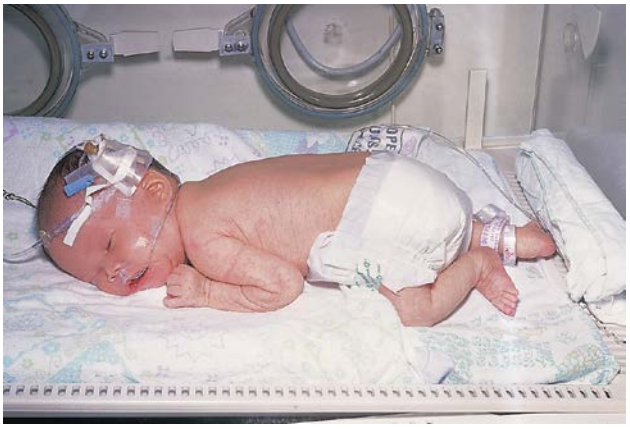

Figure 45.7 Baby girl N died of intractable acidosis in the first week. She had enormous polycystic kidneys.

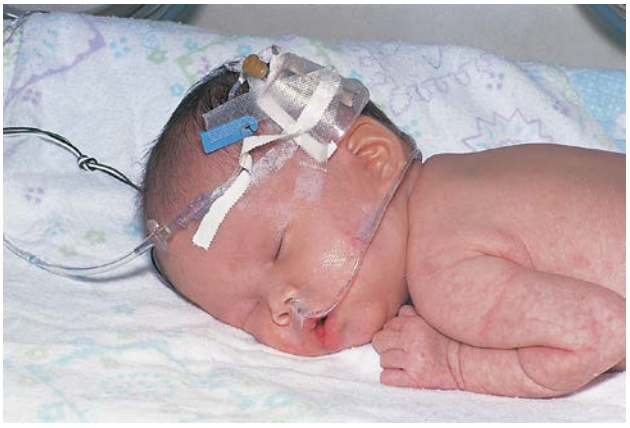

Figure 45.8 Baby girl N was not strikingly dysmorphic. She did have anteverted nares and a triangular-shaped mouth. The ears appeared low.

Infants without dysmorphic features and abnormal organogenesis have also presented early in life with tachypnea, acidosis, hypoglycemia, and an abnormal odor. Many have had hepatomegaly. Some of these have survived the initial episode and died a few months later, often with cardiomyopathy. A small number have survived a bit longer and had episodic illness reminiscent of Reye syndrome [45–47]. Sudden life-threatening disease in infancy was reported [48] in three infants with this disease, two of whom died. One had documented arrhythmia and the authors attributed the episode to myocardial dysfunction in the others.

The later onset multiple acyl CoA dehydrogenase deficiency, or ethylmalonic-adipic aciduria has presented with a considerable variety. The first patient reported [49] had episodic vomiting, hypoglycemia, and acidosis from seven weeks of age. Another [50] presented first as a 19-year-old in hypoglycemic coma and continued to have episodes of nausea, vomiting, hypoketotic hypoglycemia, and hepatic dysfunction with elevated bilirubin and transaminases, but normal ammonia. Two sisters had died in childhood of the same disease. Others have had such episodes beginning in the first year of life [51–53], but one woman presented at 25 years with a history of episodic muscle weakness and vomiting [54].

An adult patient [50] had muscle weakness and low levels of carnitine in muscle. Others had lipid storage myopathy and systemic deficiency of carnitine [55–57]. During acute episodes, these patients have had hypoketotic hypoglycemia, acidosis and sometimes, especially early in life, hyperammonemia. Transaminase levels in blood may be elevated, and there may be prolongation of prothrombin or partial thromboplastin times. Lactic acidemia may be impressive. In Taiwanese patients with mutations in the *ETFDH* gene, the phenotype was myopathic but highly variable [57]. At one extreme, a ten-year-old girl developed progressive weakness of proximal muscles and died of cardiopulmonary failure, acidosis, hypoglycemia, and hyperammonemia; on the other hand, a 27-year-old woman with exercise intolerance since childhood had episodes of pancreatitis, elevated creatine kinase, and lipid droplets in biopsied muscle. Her older sister had an even milder phenotype. Both responded well to riboflavine and carnitine.

A few patients developed a progressive extrapyramidal movement disorder [58]. Uziel and colleagues described a boy with gradually progressive spastic ataxia and leukodystrophy without ever having experienced episodic metabolic crises [59]. One adult patient presented for several years with cyclic vomiting and was initially diagnosed with cyclic vomiting syndrome [60]. A depressive state and intermittent nausea were the first symptoms of an adolescent patient with late-onset riboflavin-responsive MADD [61]. Brain magnetic resonance imaging of this patient showed disseminated high-intensity areas in the periventricular white matter and in the splenium of the corpus callosum on T2-weighted images and fluid-attenuated inversion-recovery images before starting the treatment. In summary, late-onset MADD is characterized by a progressive myopathy of varying degrees and time course, risk of acute deteriorations and metabolic decompensation, and, occasionally, additional neurologic symptoms.

Roentgenograms of the chest may reveal cardiomegaly, and echocardiography may be consistent with cardiomyopathy. Neuroimaging may reveal areas of increased signal on T_2 of the magnetic resonance image (MRI) in the basal ganglia (Figure 45.9) [62] or hypomyelination [63]. A macrocephalic patient was found to have symmetric hypoplasia of the temporal lobes of the brain in the first week of life [63]. He had normal psychomotor development for 11 months when he died of a sudden cardiac arrest. Autopsy showed hypomyelination and systemic hypoplasia of temporal lobes with loss of axons and focal subcortical ganglionic heterotopia, consistent with aberrant intrauterine developmental origin.

The diagnosis in all forms of multiple acylCoA dehydrogenase deficiency has usually been made on the basis of the unusual pattern of organic acid excretion

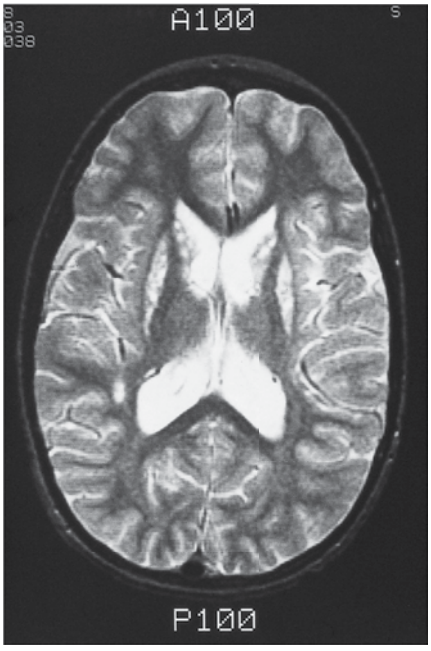

Figure 45.9 MH: Neuroimaging (magnetic resonance imaging) of the brain reveals hyperlucency of the striatum as well as an extraordinary pattern of increased T_2 signal in the white matter.

in which a large number of organic acids are found in elevated amount in the urine. This is especially true in the severe neonatal onset form in which the quantities found are enormous. The most prominent of these are lactic acid and glutaric acid, but a large number of other dicarboxylic acids and hydroxy acids are found. Among the former are ethylmalonic, adipic, suberic, and sebacic acids, as well as unsaturated suberic acids. Among the latter are 2-hydroxybutyric, 2-hydroxyglutaric, and 5-hydroxyhexanoic acids. 3-Hydroxyisovaleric and 2-hydroxyisocaproic acids are also found in the urine. Most of the same organic acids are found in increased amounts in the plasma. The concentrations of glutaric and lactic acids are prominent. p-Hydroxyphenyllactic acid may be elevated in the blood and the urine, possibly an index of immaturity or of hepatic disease.

Volatile acids are demonstrable in the plasma by analysis with gas liquid chromatography. The concentrations of isovaleric, acetic, isobutyric, 2-methylbutyric, butyric, and propionic acids may all be elevated to values 60–4800 times normal. In our first patient, the concentration of isovaleric acid was 0.76 mmol/L [34]. This would account for the odor. Elevated concentrations of these compounds are also found in the urine. Isovalerylglycine is found in the urine, as is N-isovalerylglutamic acid [46]. Acylcarnitine profiles reveal multiple esters of organic acids (Figure 45.10).

The organic aciduria is not nearly so pronounced in the milder or episodic forms of the disease. Some only manifest increased excretions of ethylmalonic and adipic acids [49]. In others, abnormal quantities of organic acids are found only during acute episodes of illness. The excretion of 2-hydroxyglutaric acid is a useful marker for this disease. This contrasts with glutaryl-CoA dehydrogenase deficiency (Chapter 9) in which 3-hydroxyglutaric acid is the hydroxy acid found.

In the neonatal onset disease, the concentrations of the amino acids, citrulline, lysine, ornithine, and proline are elevated in plasma and urine. Hydroxyproline excretion may be high, consistent with a generalized amino aciduria. The excretion of arginine may also be very high. In the later onset disease, there may be elevated concentrations of sarcosine in blood and urine [37, 40, 45]. Concentrations of carnitine in the blood may be low [57].

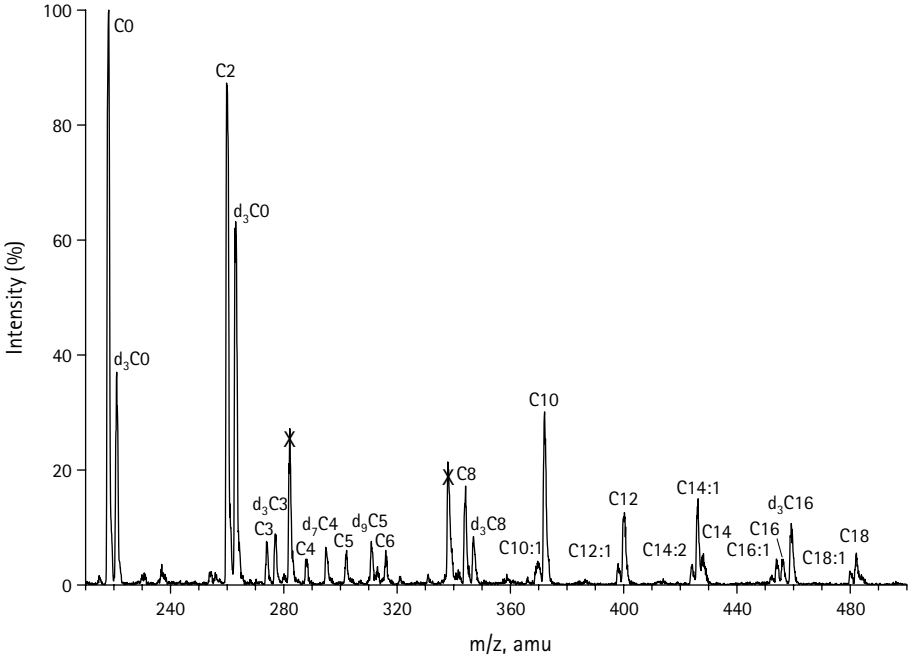

Figure 45.10 Acylcarnitine profile of the blood plasma of a patient with multiple acyl CoA dehydrogenase deficiency. The pattern of elevation of many acylcarnitine esters follows a curve with the maximum at C10. The two peaks marked X were artifacts. The elevated C0 indicated treatment with carnitine. (Illustration provided by Jon Gangoiti of University of California, San Diego.)

GENETICS AND PATHOGENESIS

The disease in each of its forms is autosomal recessive. Intermediate activities of enzymes have been documented in parents of a patient in whose fibroblasts ETF-QO was deficient [6, 7] and in parents of a patient with a mild variant [49].

Prenatal diagnosis has been accomplished by the demonstration of large amounts of glutaric acid in amniotic fluid [64]. It has also been done by documenting impaired oxidation of [46, 47, 65] substrate and by immunochemical assay [66] in cultured amniocytes.

In addition to clinical heterogeneity, heterogeneity has been observed in differing amounts of ETF and ETF-QO activity and antigen in different cell lines [67, 68]. In some, this has correlated with clinical severity [6], but in others it has not. Deficiency of ETF-QO has been found in the patients with anomalies and polycystic kidneys [40]. Deficiency of ETF-QO antigen was first demonstrated [40] in liver mitochondria of such an infant; it was also demonstrated in fibroblasts of this patient [7], and two others with renal cysts [69]. ETF-QO was nearly completely deficient in these patients, while the deficiency was less severe in patients with the later onset variants [6, 57].

ETF deficiency was found by immunoblot analysis in fibroblasts of two neonatal patients with no congenital abnormalities [7]. In one, the α- and β-subunits were both deficient, and the α-subunit was of smaller size. In the other patient, the α-subunit was also small, while the β-subunit was of normal size. The biosynthesis of the α-subunit precursor in the first cell line was virtually absent; in the second an α-subunit was made that was about 1 kDa smaller than usual. In another patient with severe disease and no anomalies, there were two weak bands of α-ETF, one smaller than normal [70]. ETF activity was virtually completely absent in these cells and in another severely ill newborn; some residual activity was found in a few patients with milder disease [5, 6]. In three patients, β-ETF deficiency was found by immunochemical assay [6, 71]. In cell lines of some patients with multiple acyl-CoA dehydrogenase deficiency with ETF and ETF-QO, activity was normal [72], raising the possibility of fundamental defects not yet discovered.

Complementation studies clearly distinguished cells of this disease from those of isovaleric acidemia [73] and provided evidence of two groups of patients with severe multiple acylCoA dehydrogenase deficiency.

Molecular analysis of the coding sequence of six patients with neonatal onset multiple acylCoA dehydrogenase deficiency revealed seven different mutations in the α-subunit of ETF [30, 74]. The most common was a substitution of methionine for threonine at codon 266, which was found in four unrelated patients. A valine 157 to glycine change has also been reported in two patients [30, 31], and a glycine 116 to arginine [30]. Three deletions were observed [30], as well as a deletion of the consensus G 5-prime slice site donor leading to an 81-bp deletion and a 26 amino acid deletion were found [29] in the late onset form (D128N).

In ETF-β mutations were found [29] in the late onset form (D128N). In Japanese brothers, compound heterozygosity was found for an arginine to glutamine change at 164 and a G to C change at the first nucleotide of the intron donor site leading to a deletion of 53 amino acids, the other allele carried a G-to-A transition at nucleotide 518, causing a change of codon 164 from arginine to glutamine [75].

In the ETF-QO, an A84T mutation was found in four Taiwanese families [57]. In an infant with the neonatal onset phenotype with congenital anomalies, a homozygous 1-bp deletion of 36A was found which led to a frameshift at alanine 12 and a stop codon at amino acid 19 [29].

In the presence of defective activity of ETF or ETF-QO, the activities of at least 9 dehydrogenases are impaired. This has most commonly been demonstrated by the incubation of fibroblasts derived from the patient with ^{14}C-labeled substrates and measuring their conversion to $^{14}CO_2$. Substrates used have included labeled glutaric acid, valine, leucine, isoleucine, 2-oxoisovaleric acid, and 2-oxoisocaproic acid [1], as well as labeled lysine, palmitic acid, butanoic acid, and butyric acid.

The conversion of 1,5-^{14}C-glutaryl CoA to $^{14}CO_2$ has been assessed in the absence of artificial electron acceptor as an assay for the presence of active ETF and ETF-QO [76]. Assay for tritium release from ^{3}H-labeled palmitic acid is also deficient in fibroblasts of patients, and this assay has been employed in complementation studies [77].

Riboflavin responsiveness has been demonstrated by the study of the oxidation of ^{14}C-labeled substrates in fibroblasts cultured in the presence and absence of riboflavin supplemented media [3]. Defective oxidation was restored to normal levels by growth in the presence of riboflavin. After growth in riboflavin-depleted medium, the level of the patient's ETF activity fell to 59 percent of control, as did the level of ^{14}C-FAD-labeled by growth in ^{14}C-riboflavin. This is consistent with evidence of clinical and biochemical responsiveness to riboflavin [78] in the patient whose cells were studied.

The clinical phenotype of MADD is influenced by environmental factors such as cellular temperature. This was particularly apparent in patients with milder forms. Overexpression studies of a β-ETF missense mutation (D128N) identified in a patient with myopathic disease showed that the residual activity of the mutant enzyme could be restored up to 59 percent of that of wild-type activity when β-ETF(D128N) transformed *E. coli* cells were grown at low temperature. The β-ETF(D128N) mutant enzyme displayed significantly decreased resistance to thermal inactivation compared to wild-type. The authors concluded that fever may lead to a further decrease in the level of active ETF enzyme activity [29].

Among the consequences of multiple acylCoA dehydrogenase deficiency is depletion of body stores of carnitine. In a later-onset patient with even relatively

mild disease, carnitine deficiency may be expected. An adult-onset patient was reported to have low levels of free carnitine in liver and muscle [50]. In a neonatal-onset patient, free-carnitine levels in the blood may be low, but they may be normal; however, carnitine esters in the urine are high [79, 80]. This excretion of esters is increased after treatment with carnitine [39]. Specific carnitine esters identified include acetylcarnitine, isobutrylcarnitine, isovalerylcarnitine, hexanoylcarnitine, propionylcarnitine, and butyrylcarnitine. Rapid diagnosis may be made by the analysis of acylcarnitine profiles in plasma or blood spots on filter paper [81, 82], and the disease is detectable in programs of newborn screening. In this disease, there is a general accumulation of acylcarnitines from C4 to C18 [81]. It has also been documented that this approach to diagnosis may miss a patient with mild MAD deficiency [81].

TREATMENT

The treatment of the neonatal-onset patient is supportive, especially the treatment of acidosis, hypoglycemia, and dehydration with huge amounts of appropriate fluids. Nevertheless, most of those with polycystic kidneys die promptly.

Later-onset patients, and those who survive the initial episode, should be assessed for riboflavin responsiveness, reported to be best judged by changes in the dicarboxylic aciduria. Most patients are treated with riboflavin in doses of 100–300 mg/24 hours [52, 53, 78, 83], as well as carnitine. A riboflavin-responsive boy was reported to develop progressive spasticity, ataxia, and leukodystrophy without ever experiencing acute metabolic imbalance [59]. Improvement after treatment with riboflavin and carnitine has mainly been reported in patients with mutations in the *ETFDH* gene [57]. Restriction of the intake of fat and protein may be prudent, dependent on the severity of the disease. Dietary management must maintain caloric intake while limiting the load of affected amino acids and providing only the minimum of long-chain fats to keep the patient from becoming essential fatty acid depleted. It is usually recommended that 65–75 percent of total energy intake come from carbohydrates, 20–25 percent from fat (including essential fatty acids), and 8–10 percent from proteins. The goal is to provide sufficient glucose to stimulate insulin secretion to levels that will suppress fatty acid oxidation in liver and muscle and will block adipose-tissue lipolysis. A fat-restricted diet may put patients at risk of deficiency of essential fatty acids; therefore, supplementation with essential fatty acids can be necessary in order to meet the requirements for age (1–4% of energy intake). Glycine supplementation may also remove accumulated CoA esters as their glycine conjugates, as in isovaleric acidemia (Chapter 8). In a nine-year-old patient with milder disease, glycine supplementation was as effective as carnitine supplementation in handling a medium-chain triglyceride (MCT) load [84]. Inasmuch as the major conjugated compounds excreted after glycine are different from those after carnitine, it would be prudent to treat patients with both. Some patients may be able to tolerate fasting periods up to 12 hours. Determination of an individually safe fasting tolerance should be done under controlled circumstances and careful clinical supervision, and it should include the determination of plasma acylcarnitine profiles and urinary organic acids in short intervals [85].

Differential diagnosis: riboflavin transporter defect

Three patients were reported [86] who had progressive muscle weakness and paralysis of the diaphragm in whom patterns of acylcarnitine profiles and urinary organic acids suggested an attenuated form of multiple acylCoA dehydrogenase deficiency. They were found to be deficient in riboflavin and also of flavinmononucleotide and FAD. Levels of riboflavin were restored by treatment with riboflavin and clinical manifestation improved markedly. Mutations were found in the *C20orf54* gene which encodes the human homolog of the rat transporter for riboflavin. The first two patients were homozygous c.1198-2A>C, an acceptor splice-site mutation; the other patient was heterozygous for p.W17R and p.Y213X. Mutations in this gene were independently found [87] in patients with the Brown–Vialetto–Van Laere syndrome (MIM 211530) and its allelic variant Fazio-Londe syndrome (MIM 211500). Both conditions are motor neuron syndromes which respond well to high-dose (10 mg/kg per day) riboflavin supplementation.

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46

3-Hydroxy-3-methylglutarylCoA lyase deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, metabolic acidosis, hyperammonemia; hepatomegaly; a characteristic organic aciduria: 3-hydroxy-3-methylglutaric, 3-methylglutaconic, 3-methylglutaric, and 3-hydroxyisovaleric acids; and deficiency of 3-hydroxy-3-methylglutarylCoA lyase.

INTRODUCTION

3-Hydroxy-3-methylglutaric (HMG) aciduria is a disorder of leucine metabolism (Figure 46.1) that leads to life-threatening illness early in life. Once diagnosed, management, particularly the avoidance of fasting, can be very rewarding. The first patient was reported in 1976 by Faull and colleagues [1]. This infant was well until seven months, when he developed diarrhea and vomiting, and within 24 hours he had lethargy, pallor, dehydration, cyanosis, and apnea, requiring resuscitation. At four years and seven months [2] development was satisfactory. The disorder has been encountered frequently among Arab families [3].

HMG-CoA lyase deficiency may be considered an organic acidemia. It is, at the same time, a classic disorder of fatty acid oxidation. HMG-CoA lyase is the last step in the formation of acetoacetate (Figure 46.2) and its product, 3-hydroxybutyrate. The products of the cleavage of HMG-CoA are acetoacetate and acetylCoA. HMG-CoA is also of course a key intermediate in the synthesis of cholesterol (Chapter 84). Its reduction to mevalonic acid represents a feedback control point in this pathway.

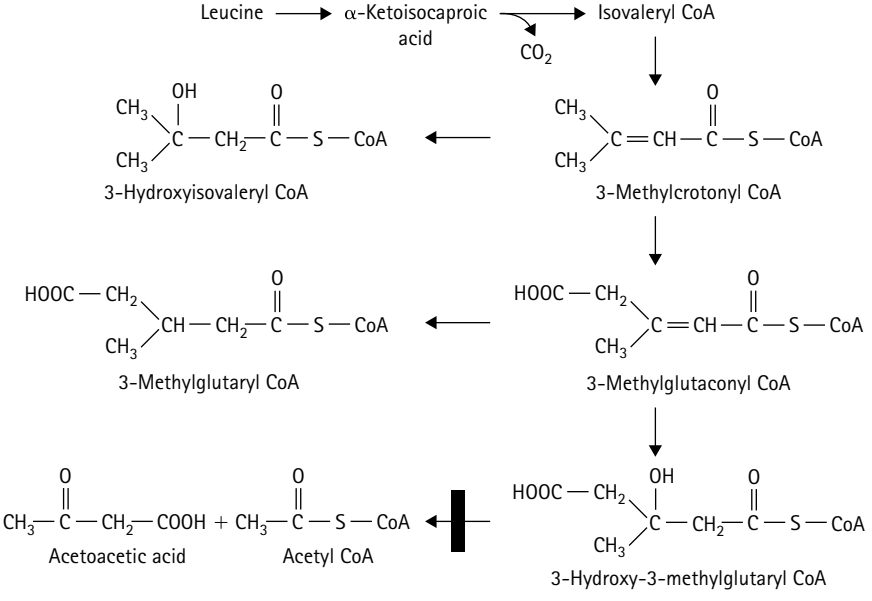

Figure 46.1 The pathway of the catabolism of leucine and HMG-CoA lyase, the site of the defect in HMG aciduria.

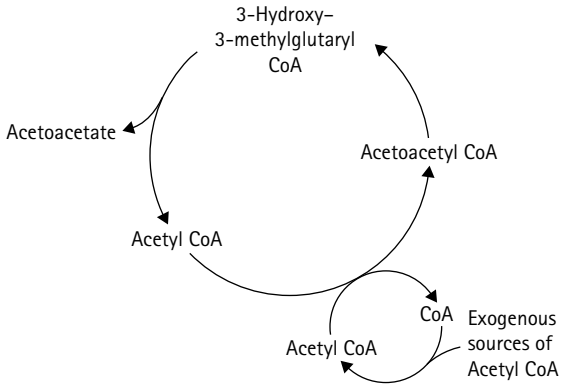

Figure 46.2 Ketogenesis. HMG-CoA and its lyase play a critical role.

CLINICAL ABNORMALITIES

The classic presentation is with a Reye-syndrome-like episode in late infancy (from six months to two years), usually following an intercurrent infection which leads to vomiting or failure to eat [1–8]. Some present in the neonatal period, but the majority between three and 11 months. The disease has also been reported to clinically manifest first in adults. A 36-year-old woman with seizures, and severe leukoencephalopathy, and a 29-year-old adult with no prior history of the disease have been reported [9, 10].

Persistent vomiting may be an early symptom. There is rapid progression from lethargy and hypotonia to coma. Pallor and dehydration are commonly present. There may be seizures, including myoclonus. Hypothermia has been reported [11]. Apnea is followed by death unless the patient is artificially ventilated. Clinical chemical evaluation reveals hypoglycemia, metabolic acidosis, and, in some, hyperammonemia. For this reason, a number of patients have initially been diagnosed as Reye syndrome [6]. Presentation with life-threatening acidosis is common [4, 8, 11]. Infants may present in the first days of life with seizures, lethargy, or tachypnea. This may follow the first feeding or may precede it, an index that birth itself maybe a catabolic experience. Lactic acidemia maybe prominent. The initial episode may be fatal [8, 11].

Recurrent episodes of acute illness have been observed particularly in those who presented in the neonatal period [8]. The patient is always at risk of acute illness, if infection or another problem leads to fasting. Some families have learned to intervene sufficiently, promptly, and effectively that episodes have been prevented or aborted.

Hepatomegaly is a regular occurrence [12], and there may be elevation of levels of transaminases in the blood. However, hepatomegaly may be absent especially in the neonatal presentation. It has been absent in a 9-month old, who had elevated transaminases [13]. Histologic examination of the liver reveals infiltration of fat.

Brain injury may result from hypoglycemia, shock, or both. Some patients have had a persistent seizure disorder

Figure 46.3 An eight-year old girl who was diagnosed as having HMG-CO lyase deficiency in the first month of life when she presented with severe hypoglycemia (blood sugar was 0.5 mmol/L), negative urine for ketones and convulsions. She required anti-convulsant medications.

and abnormalities of the electroencephalogram (EEG) [6, 8] [Figure 46.3]. Microcephaly has been observed in several patients [14, 15]. One severely retarded patient was macrocephalic [16]. Hemiparesis has been reported [17] and decerebrate tetraparesis [8]. Mental retardation may be severe [8, 15]. On the other hand, most patients are developmentally normal. There are no dysmorphic features (Figures 46.3–46.9).

One patient (Figure 46.6) presented at five years with pernicious vomiting and abdominal tenderness and was found to have acute pancreatitis [19]. She had recurrent episodes of hypoglycemia and acidosis. Pancreatitis has been described increasingly in patients with Reye syndrome [20] and in inborn errors of metabolism [21], suggesting further commonalties in pathogenesis of other metabolic disorders thought to be Reye syndrome.

Magnetic resonance imaging (MRI) of the brain reveals increased T_2 signal indicating hypodensity of white matter [8, 13, 16] (Figures 46.10, 46.11). In patients in whom brain damage has occurred, the picture may be that of cerebral atrophy [8,18] (Figure 46.12).

The hypoglycemic acute episode is striking, often extreme and with a notable absence of ketosis. Initial concentrations of sugar in the blood have ranged from 0.2 to 1.8 mmol/L; many were below 0.4 mmol/L (8 mg/dL) and one patient died in a second episode of hypoglycemia in which the concentration recorded was less than 0.1 mmol/L. At this time, the concentration of insulin was less than 1 mU/L. The episode had followed a change in diet in which the amounts of leucine ingested were increased. The infant developed cyanosis, vomiting, and hypotonia. In three patients, the values for the blood pH were recorded as 7.24, 7.11, and 7.29 [6, 7, 11]. During the acute crisis, a pH below

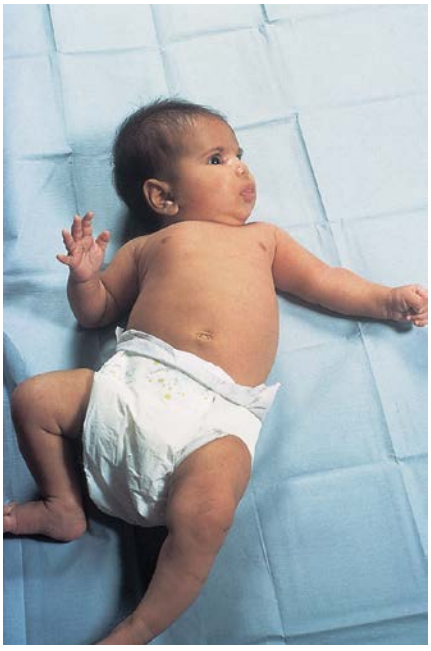

Figure 46.4 HHHA: a one-month-old infant with HMG-CoA lyase deficiency. She developed lactic acidosis and coma at days days-of-age and was found to have 668 mmol/creatinine of HMG in the urine. Activity of HMG-CoA lyase in fibroblasts was 0.9 percent of control. The tonic neck reflex is normal at her age. Examination was unremarkable.

7.0 is not unusual especially in the neonatal onset patients [4, 8]. The initial plasma concentrations of bicarbonate were below 16 mEq/L in five patients [4]. Lactic acidosis has been documented with levels as high as 10 and 20 mmol/L [4]. Persistent infantile hypoglycemia in the presence of metabolic acidosis is an indication for organic acid analysis.

Hyperammonemia has been observed in about 50 percent of patients [4]. In three patients, concentrations ranged from 388 μM/L to 1370 μmol/L [4] and in one patient the plasma concentration of ammonia was greater than

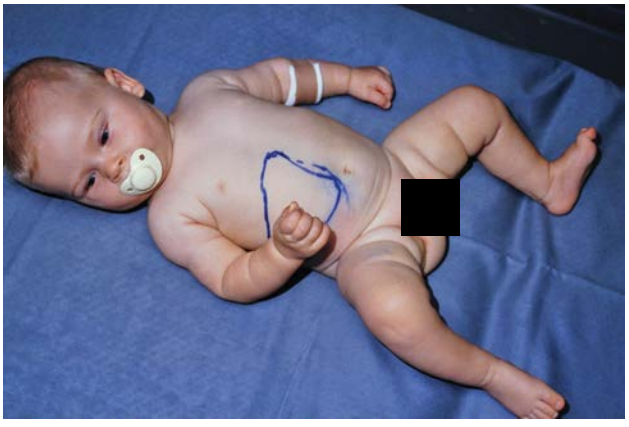

Figure 46.5 An infant with HMG-CoA lyase-deficiency who was admitted the previous night with a hypoglycemic seizure. After correction he appeared well. The enlarged liver receded with treatment over months.

Figure 46.6 A girl with 4 hydroxy-3-methylglutaric aciduria. She was admitted to hospital at five years of age with acute pancreatitis. The activity of 3-hydroxy-23-methylglutaryl CoA lyase in lymphocytes and fibroblasts was 2 percent of normal. (The illustration was kindly provided by Dr William Wilson of the University of Virginia.)

2000 μmol/L [3]. Abnormal liver function tests included alanine and aspartate aminotransferase [1, 6]; bilirubin, gamma-glutamyl transpeptidase (gGT) [11], and prolonged prothrombin time [6, 7], all of which lead to confusion with the diagnosis of Reye syndrome. In fact, the provisional diagnosis was Reye syndrome in the first four patients reported. With successful treatment, the abnormalities in liver function disappear. The prognosis is guarded. Death

Figure 46.7 A three-year-old patient with HMG-CoA lyase deficiency. She developed severe neonatal lactic acidosis, hypoglycemia and coma. Despite noncompliance with dietary treatment and multiple acidotic attacks she was developing normally. By follow-up at seven years of age, she was doing better than average in school though attacks were continuing.

Figure 46.8 S: A three-year-old girl with HMG-CoA lyase deficiency. She had severe lactic acidotic episodes in the neonatal period and at 5 and 14 months, in the latter of which she developed shock and convulsions along with an unmeasurable blood glucose. On follow-up at 7 years-of-age, she had no attacks for three years and was developing normally.

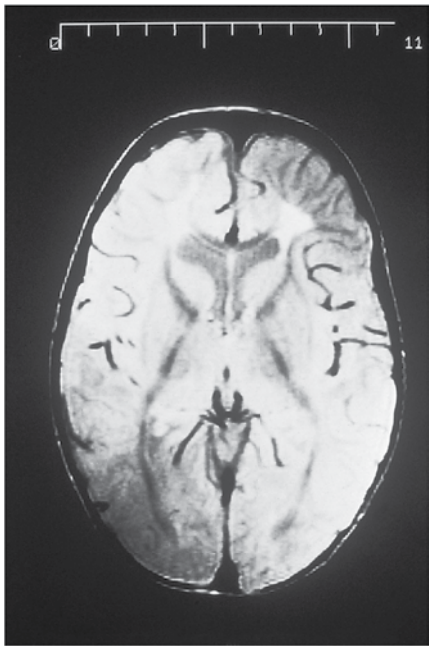

Figure 46.10 MRI of the brain of the patient in Figures 46.3 and 46.4 at 2.5 years revealed increased signal intensity in the frontal white matter. (Reprinted with permission from the Journal of Inherited Metabolic Disease [9].)

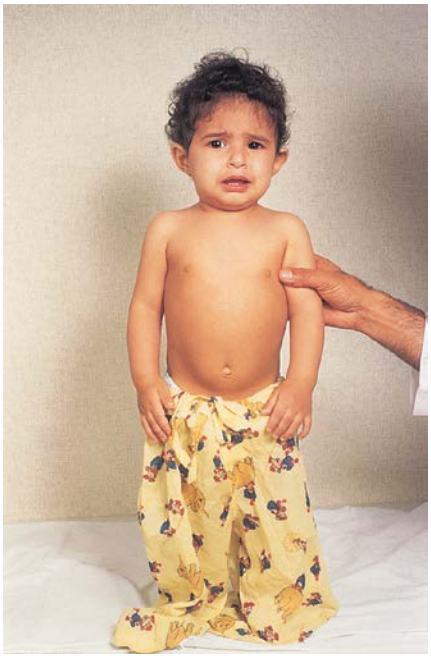

Figure 46.9 A 21-month-old boy with HMG-CoA lyase deficiency. His first acidotic episode was at 6 months-of-age following herpangina and refusal to eat. At 6 years-of-age, he had not had an attack in three years and was doing very well in school.

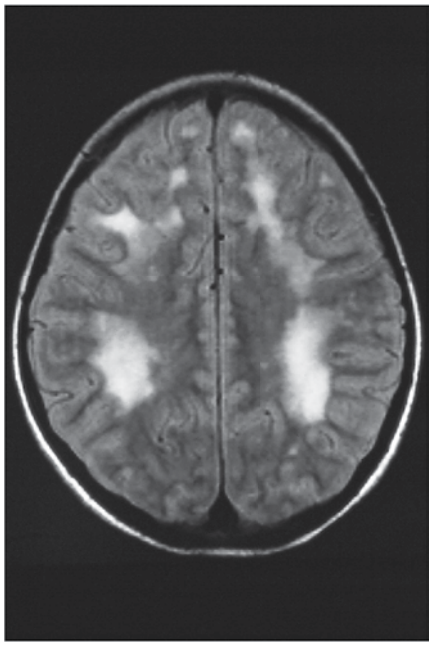

Figure 46.11 MRI scan of the brain of a nine-year-old with HMG-CoA lyase deficiency. There was extensive increase in signal in the subcortical white matter consistent with dysmyelination. (Kindly provided by Dr Robert Schwartz of Brown University School of Medicine.)

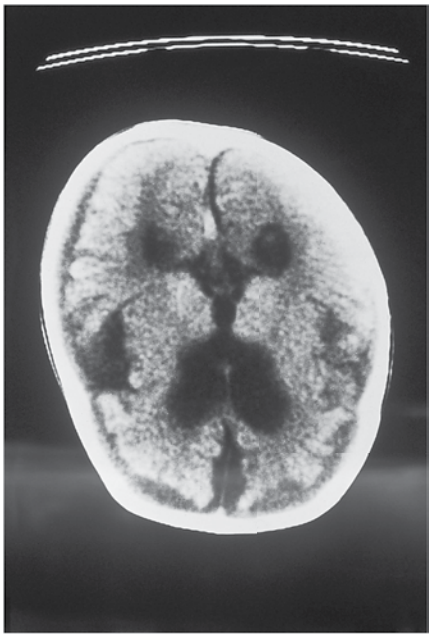

Figure 46.12 The sister of the patient in Figure 46.7. She was diagnosed late. The CT scan at 10 months shows considerable evidence of cerebral atrophy. She was severely impaired neurologically and died at 19 months.

has been observed in at least five patients [8, 11, 17, 18, 22], as has sudden death in a 13-month old.

None of these patients had ketonuria at times of acute illness, and in some low levels of acetoacetate and 3-hydroxybutyrate have been documented in the blood [7, 17, 18]. Levels of acetoacetate and 3-hydroxybutyrate in the urine are disproportionately low [22]. This serves to distinguish these patients from those with other organic acidurias, such as propionic acidemia (Chapter 2) or methylmalonic acidemia (Chapter 3). This is consistent with the site of the defect (see Figure 46.2) in which HMG acid cannot be converted to acetoacetic acid and acetylCoA. Ketone bodies decrease proteolysis in muscle and conserve muscle protein during starvation [23]; so, impairment of ketogenesis could be relevant to the hyperammonemia.

GENETICS AND PATHOGENESIS

HMG-CoA lyase deficiency is transmitted as an autosomal recessive trait. Consanguinity has been observed in a number of families [6, 8, 11]. The disease is uncommon except in Saudi Arabia [3], where it was documented in 16 percent of organic acidemias. From Japan, five patients were reported [24].

The molecular defect is in the enzyme HMG-CoA lyase (see Figure 46.1). Defective activity of the enzyme has been demonstrated in cultured fibroblasts [25, 26], leukocytes [27], and liver [11] of affected patients. Activity in 10 patients was reported to be undetectable; in 16 others, it ranged from 0.7 percent to 13.7 percent of normal [4]. A variety of methods is available for enzyme analysis [28], including direct detection by high performance liquid chromatography (HPLC) of the product of the reaction [26]. In addition, the defect can be identified by measuring the incorporation of ^{14}C-isovaleric acid into trichloroacetic acid perceptible macromolecules [29] or by monitoring metabolism of ^{14}C-leucine [30].

Mutation at Arg41 was found in a patient with severe deficiency of the enzyme [31] which is consistent with a role for interaction of Arg41 with the acylCoA carbonyl, promoting product enolization.

The crystal structure of HMG-CoA lyase has been published [32]; it has confirmed barrel structure predicted by molecular modelling [32, 34] in which there is a carboxyl end cavity formed by eight-β-strands of the barrel, which have one molecule of 3-hydroxyglutaric acid and Mg++. Substrate binding involves asparagine 311 and lysine 313 and the establishment of polar contacts with phosphate and ribose groups of adenosine [32]. A G-loop structure would facilitate a disulfide bond between cysteine 266 and cysteine 323 [31].

The activity of the HMG-CoA lyase in leukocyte or fibroblasts in the parents of patients is intermediate between those of the patient and controls [4, 26, 27]. However, in some families, obligate heterozygotes have had normal values, study of expression at the levels of mRNA protein and enzyme activity revealed greatest activity in the liver [34], followed unexpectedly in various tissues. In the heart and adult brain, activity was not detected. These findings are consistent with the occurrence of pancreatitis in this disease. The striatum has been viewed as particularly vulnerable to oxidative damage by 3-hydroxy-3-methylglutarate which is a strong pro-oxidant [35].

Prenatal diagnosis has been accomplished by the analysis of metabolites in maternal urine at 23 weeks of gestation [36]. The enzyme is active in amniocytes [37]. Prenatal diagnosis should be possible by direct measurement of HMG by stable isotope dilution gas chromatography/mass spectrometry (GC/MS) of the amniotic fluid.

The gene for HMG-CoA lyase has been cloned [38]. The sequence predicts a 27-residue mitochondrial leader and a 31.6 kDa mature protein. A number of mutations have provided interesting information on the nature of the enzyme. Five mutations in the highly conserved R41 and D42 codons were found in 23 percent of the mutant alleles in 41 probands. They were R41Q, R41X, D42H, D42G, and D42E [39]. R41Q is common in Saudi Arabia where six of nine probands were homozygous. This mutation has also been found in Turkish and Italian patients. In three Czech patients, two known missense mutations were found along with a novel one base deletion 27 delG in exon 1 [40]. Among major alterations in the gene were two large deletions [41] and three frameshift/premature terminations [38, 39]. Two stop codon mutations and a two base pair deletion led to alternately spliced mRNA [42–44]. Among 93 patients reported, 30 variant alleles [33] were found

(28 mutations and 2 SNPs). Particular frequency was noted in Saudi Arabia and the Iberian Peninsula where two mutations (122G>A and 109G>A) have been identified in 87 percent and 94 percent. Some clustering was observed in exon 2 [33].

In Brazil, two mutations were predominant in ethnic Portuguese/Spanish (E37X and V168fs (-2)) [45]. A single (E37X (c.109G>T) was found in 84 percent of alleles from Northern Portugal [46]. In Taiwanese patients [47] c.494G>T, p.Arg165Gin and two splice-site mutations (IVS3+1G>A and IVS6-1G>A) leading to skipping of exon 3 were predominant. Despite these elegant studies, a clear relationship of genotype to phenotype has not emerged and it is possible that this reflects the fact that clinical manifestations are more likely the result from hypoglycemia and its consequences than from severity of medication in enzyme activity.

The pattern of organic aciduria in this disorder is characteristic [48–51] (see Figure 46.13). HMG acid is excreted in appreciable quantities in the urine. In acute crisis, levels may reach 10,000 to 20,000 mmol/mol creatinine and between crises may be 200–4000. Normal individuals excrete in urine less than 100 mmol/mol creatinine [49]; levels are somewhat higher in young infants. Organic acid analysis by GCMS usually involves trimethylsilyl derivatives, but in the case of HMG acid, appreciable quantities of the di-derivative are formed as well as the tri-derivative, and both must be included for quantification [52]. Large amounts of 3-methylglutaconic acid are also found in the urine. These compounds represent successive steps in the catabolism of leucine (see Figure 46.1) in which isovalerylCoA is converted to 3-methylcrotonyl CoA which is then converted to 3-methylglutaconylCoA. The addition of H_2O across the double bond in this compound yields 3-hydroxy-3-methylglutarylCoA, which is ultimately cleaved to form acetoacetic acid and acetylCoA. 3-Methylglutaric acid is also found; this would result from reduction of 3-methylglutaconic acid. The reaction could be catalyzed by the enzyme that catalyzes the reverse dehydrogenation of 3-methylcrotonylCoA to isovalerylCoA. In addition, 3-hydroxyisovaleric acid is also found in the urine [51]. This compound would arise from the hydration of 3-methylcrotonyl CoA. In the acute episode a large elevation of 3-hydroxyisovaleric acid may be found, along with 3-methylcrotonylglycine [19, 51–53]. Glutaric acid and adipic may also be elevated in the urine in the acute crisis [54]. Lactic acid levels may be elevated at these times [55], along with hyperammonemia. Levels of acetoacetate and 3-hydroxybutyrate are disproportionately low in the urine, as they are in the blood.

3-Methylcrotonic (3-methyl-2-butenoic) acid may be found in the urine, along with its isomer, 3-methyl-3-butenoic acid [48], but these are artefacts of the GCMS analysis [54, 56]. The abnormal metabolites in this disease may also be detected by nuclear magnetic resonance (NMR) spectroscopy [57] permitting rapid diagnosis. Rapid diagnosis is now more likely to be made by tandem mass spectrometry (MS/MS). 3-Methylglutarylcarnitine has been found in the plasma and urine [58], and 3-hydroxy-isovalerylcarnitine has been found in plasma [59] (see Table 1.2). This permits the incorporation of this disease into programs of neonatal screening.

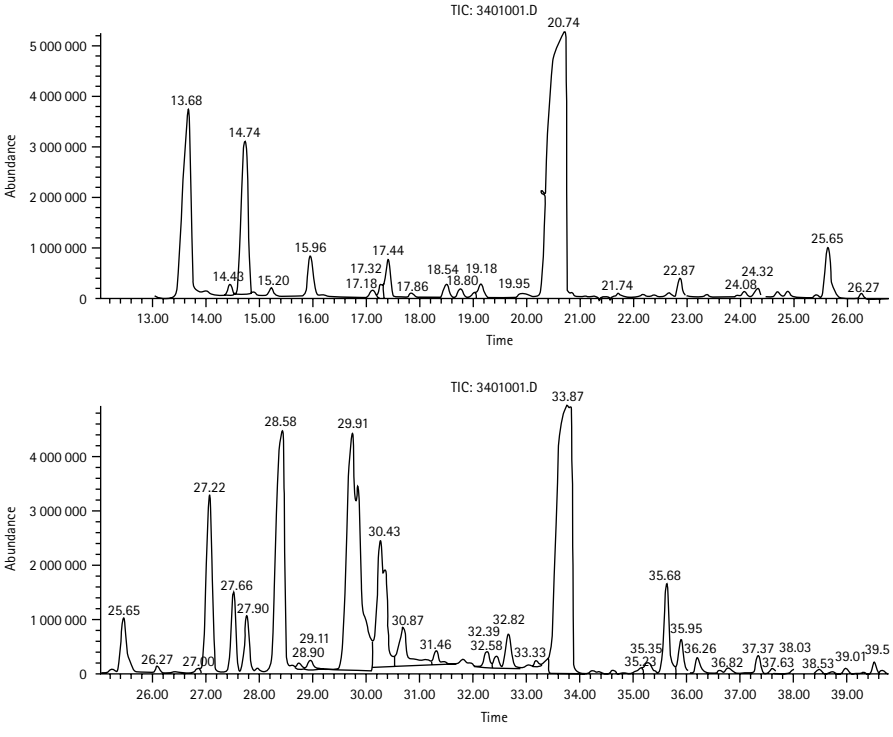

Figure 46.13 Organic acid analysis of the urine of a patient with HMG-CoA lyase deficiency. The important compounds were: 3-hydroxyisovaleric acid at 20.74; 3-methylglutaric acid at 27.90; 3-methylglutaconic acid at 28.58 and 29.91; and HMG acid at 33.87.

The excretion of acylcarnitine esters is elevated in this condition, and there may be a secondary deficiency of free carnitine [60].

TREATMENT

Management of the patient should be considered under two headings: long-term management and treatment of the acute crisis. The latter is an emergency, and care must be devoted first to measures of general support such as assisted ventilation and repair of deficits of fluid and electrolytes and elevation of the blood concentration of glucose. In an infant with or without hyperammonemia, exchange transfusion, or peritoneal dialysis may be necessary. Sodium benzoate or phenylacetate and L-arginine are useful in management of the hyperammonemia [61]. Hypoglycemia and acidosis usually respond readily to the parenteral administration of 20 percent glucose with intravenous insulin to keep blood sugar between 6 and 8 mmol/L, fluid and electrolytes. Parents should be instructed to bring the patient in early, whenever the oral route is compromised by vomiting or anorexia.

Long-term management rests largely on the avoidance of hypoglycemia, and the avoidance of long fasting, especially during intercurrent illness. The importance of ketogenesis in glucose homeostasis was illustrated by the prevention of hypoglycemia with fasting in a patient with HMG-CoA lyase deficiency by the infusion of 3-hydroxybutyrate [62]. Frequent feedings are advisable in infancy, with even sleeping through the night permitted only after it is documented that this does not lead to hypoglycemia. A high carbohydrate diet with added cornstarch is advisable; and supplementation with glucose polymers is convenient, especially during intercurrent illness. Cornstarch is useful at bed time.

Restriction of the intake of protein has been employed, and it appears prudent to restrict the amounts of leucine ingested. Restriction of the intake of fats may reduce the levels of metabolites in the urine [12]. Carnitine supplementation has proven to be a useful adjunct to therapy [63].

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PART 5

THE LACTIC ACIDEMIAS AND MITOCHONDRIAL DISEASE

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47

Introduction to lactic acidemias

INTRODUCTION

The lactic acidemias constitute a large family of distinct disorders of metabolism. There are enlarging numbers of deficiencies of enzymes, and some disorders are now characterizable on the basis of the mutation in the DNA. This is especially the case in mitochondrial DNA, but mutations are increasingly being detected in nuclear DNA. Some patients have lactic acidemia secondary to another disorder, such as propionic acidemia (Chapter 2). On the other hand, there remain a considerable number of patients with lactic acidemia in whom a molecular explanation of the abnormal metabolism cannot be found, even with the most sophisticated studies available. Elucidating the cause and the most appropriate approach to therapy in a patient with lactic acidemia requires a systematic investigation.

In considering a patient for investigation of lactic acidemia, it is first necessary to establish that elevation of lactic acid in the blood is real. This may require a number of determinations even in patients with known disease. The most common reason for elevated concentration of lactic acid in blood is improper technique, the use of a tourniquet, or a real struggle in obtaining a sample. On the other hand, levels are variable even in patients with known disease. This is a function of the fact that lactic acid itself is situated some distance from most of the known defective enzymatic steps, particularly oxidative steps in the electron transport chain. The first step is the documentation of elevated levels of lactic acid, pyruvic acid, and/or alanine in the blood. It is important to be rigorous about methods of sampling, to draw blood that is flowing freely without a tourniquet. Our best results are often obtained in the course of studies in the clinical research center in which a catheter is placed in the vein to permit multiple sampling without the stresses of venepuncture. The concentration of lactate in the cerebrospinal fluid (CSF) may also be elevated. Increasingly, patients are encountered in whom the concentration of lactate in the CSF is elevated, whereas that of plasma is normal or only slightly or intermittently elevated [1].

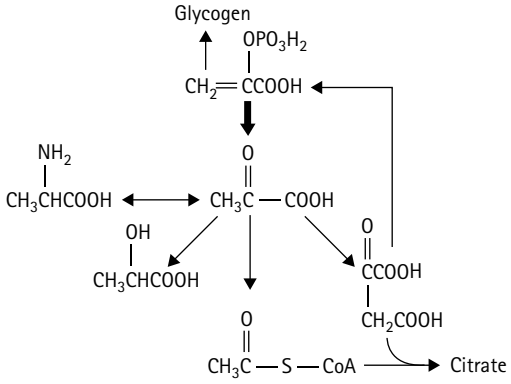

Figure 47.1 Pyruvate plays a central role in glycolysis and in oxidative metabolism. Pyruvate does not accumulate when its metabolism is blocked; it is converted to sinks or reservoirs of lactate and alanine.

The lactic acidemias are disorders of pyruvate metabolism. Concentrations of pyruvate are determined, but large elevations of pyruvate are seldom seen. Accumulating pyruvate is converted to lactate and alanine (see Figure 47.1). Concentrations of alanine are not raised factitiously by problems of technique, but they too are variable in patients with known enzymatic defects.

The next step is to exclude the conditions that lead to secondary elevations in concentrations of lactic acid. A major group of patients are those with hypoxia, hypoventilation, shock, or hypoperfusion. These situations are seen in patients with sepsis, cardiac and pulmonary disease, hepatic disease, and severe anemia. Therefore, all of these conditions should be excluded before undertaking a metabolic work up for the elucidation of a lactic acidemia. Anaerobic exercise also produces lactic acidemia, but this is seldom an issue clinically, except in the patient who has just had convulsions.

Among patients with metabolic disease, lactic acidemia is often seen, particularly at times of acute illness, as a

secondary complication of the underlying metabolic defect. These metabolic diseases include propionic acidemia (Chapter 2), methylmalonic acidemia (Chapter 3), isovaleric acidemia (Chapter 8), 3-hydroxy-3-methylglutarylCoA lyase deficiency (Chapter 46), and pyroglutamic aciduria. Lactic acidemia occurs in multiple carboxylase deficiency (Chapter 6) as a direct consequence of the defect in pyruvate carboxylase. Each of these conditions can be excluded by organic acid analysis [2–4]. Disorders of fatty acid oxidation (Chapters 34–46) may also lead to acute elevations in the concentration of lactic acid. They are best excluded by assay of the acylcarnitines of the blood (Chapter 36) [5]

WORK UP OF A PATIENT WITH CONGENITAL LACTIC ACIDEMIA

The search for mutation in mitochondrial DNA may lead directly to the molecular diagnosis. We also determine the acylcarnitine profile by tandem mass spectrometry. This might already have been done to rule out a disorder of fatty acid oxidation, but we are increasingly referred patients who are known only to have lactic acidemia, and it is convenient to carry out both assessments. This is particularly important because patients with defects in the respiratory chain can have secondary alterations in fatty acid oxidation [6, 7]. In studies in which fibroblasts were labeled with deuterated hexadecanoic acid in the presence of carnitine, a variety of patterns were observed in patients with known electron transport deficits, including the C4 pattern mimicking short chain acylCoA dehydrogenase (SCAD) deficiency (Chapter 42) and the C6, C8, C10 pattern of medium-chain acylCoA dehydrogenase (MCAD) deficiency (Chapter 39).

Examination of mitochondrial DNA should include a search for the common point mutations, as well as a Southern blot for deletions. This should yield definitive diagnoses in the case of mitochondrial encephalomyelopathy lactic acidemia and stroke-like episodes (MELAS) (Chapter 51), mitochondrial encephalomyelopathy and ragged red fibers (MERRF) (Chapter 52), neurodegeneration, ataxia, and retinitis pigmentosa (NARP) (Chapter 53), Kearns–Sayre syndrome (Chapter 54), and Pearson syndrome (Chapter 55).

Patients judged to have congenital lactic acidemia and not found to have an abnormality in mitochondrial DNA fall into two categories, those with defects in gluconeogenesis and those with defects in oxidation. It is important in the work up to distinguish clearly into which of the two categories each patient falls. The distinction may be useful in designing therapy, even in those patients in whom a molecular diagnosis remains elusive (Figure 47.2).

The central feature of this assessment (Figure 47.3) is to undertake a prolonged fast in which glucagon is given early in order to deplete the liver of glycogen made from glucose. As the fast is continued for 18–24 hours, the body must carry out gluconeogenesis in order to maintain euglycemia. At the end of the fast, this is confirmed by another glucagon test. In the absence of gluconeogenesis, the blood concentration of glucose does not rise.

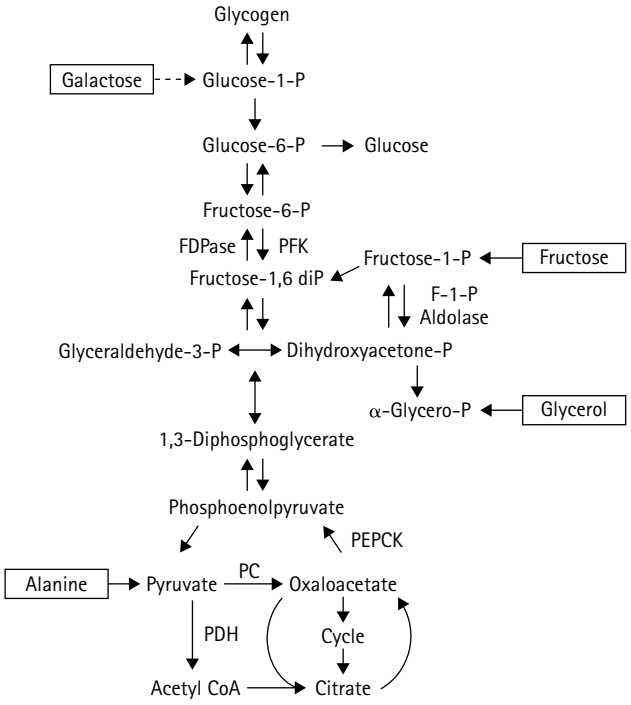

Figure 47.2 Pathways of metabolism for pyruvate through gluconeogenesis and oxidation. Boxes highlight compounds that have been used in tests to elucidate defects in gluconeogenesis.

In this procedure, an intravenous (IV) catheter is inserted to facilitate the drawing of samples. Prior to the initiation of fasting, blood is obtained for glucose, lactate, pyruvate, and alanine. After six hours of fasting, 0.5 mg of glucagon is given intramuscularly, and the glucose response is determined at 15, 30, 45, 60, and 90 minutes. The response to glucagon should be a sizable increase in glucose, except in glycogenosis type I (Chapter 59). The fast is then continued for 24 hours if the patient remains euglycemic. The blood concentration of glucose is monitored by determination at the bedside and quantitative determinations are carried out at intervals and in the presence of an abnormal test or symptoms of hypoglycemia. If hypoglycemia develops at any time, the fast is concluded and glucagon given. The IV catheter ensures the prompt IV administration of glucose to restore normoglycemia. Concentrations of lactic and pyruvic acids and alanine are obtained at the end of the fast prior to the administration of glucagon. In a hypoglycemic patient, levels of insulin, growth hormone, and glucagon are also obtained if this information is not available from prior testing.

In a patient who fails the fasting test and appears to have a defect in gluconeogenesis, it is convenient to assay biotinidase [4] in serum and carboxylases in leukocytes or fibroblasts; these procedures are noninvasive and provide a rapid answer to the diagnosis. Most patients with disorders of gluconeogenesis in whom these two procedures do not provide the diagnosis require liver

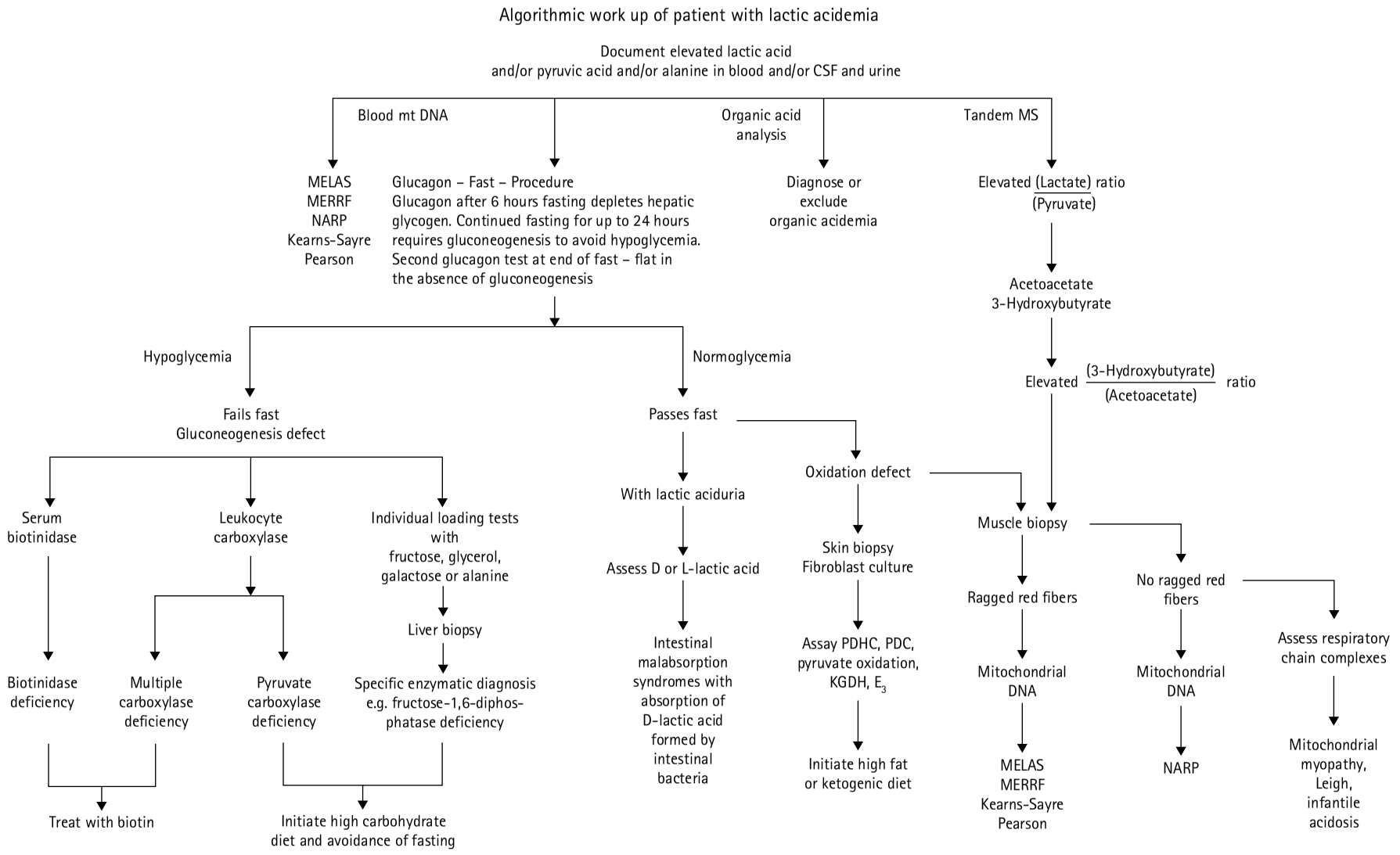

Figure 47.3 Algorithm for decision-making in congenital lactic acidemia: CSF, cerebrospinal fluid; E3, lipoamide dehydrogenase; KGDG, 2-oxoglutarate dehydrogenase; PDC, pyruvate decarboxylase; PDHC, pyruvate dehydrogenase complex.

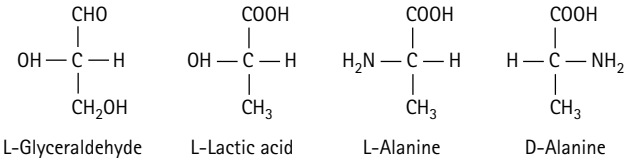

Figure 47.4 Structure of L-lactic acid and related compounds.

biopsy for definitive enzyme assay. Information as to the area of the defect may be obtained by loading tests, for instance with fructose, alanine, or glycerol in fructose-1,6-diphosphatase deficiency (Chapter 49) [8, 9]. Following glycerol or fructose, phosphate should also be measured because it decreases sharply in patients with a block at this level. Concentrations of uric acid may increase. Loading with galactose should provide a positive control except in a patient with glucose-6-phosphatase deficiency. Each compound is given by mouth as a 20 percent solution 6–12 hours postprandially in a dose of 1 g/kg.

Most patients who pass the fasting test have defects in oxidation of pyruvate. A small number has essentially factitious lactic acidemia with lactic aciduria in which D-lactic acid is formed by intestinal bacteria and then absorbed. L-lactic acid and L-alanine are actually dextrorotatory in the polarimeter, but the nomenclature is employed to indicate their structural similarity to L-glyceraldehyde (Figure 47.4). D-lactic acid and D-amino acids are bacterial components. N-Acetylmuramic acid, a compound of D-lactic acid and N-acetylglucosamine, is a component of the mucopeptides of bacterial cell walls. D-lactic aciduria is usually seen in patients with malabsorption syndromes, as well as the short bowel syndrome and necrotizing enterocolitis [10, 11]. This lactic acid accumulation can even lead to systemic acidosis and coma. A course of treatment with oral neomycin or metronidazole may resolve this problem, as may testing for lactate with an enzymatic assay specific for L-lactic or D-lactic acid. Factitious lactic acidemia and/or lactic aciduria may also occur in the neonatal intensive care unit or elsewhere when glucose is infused in amounts in excess of the capacity of the infant to utilize it [12].

In patients who pass the test and are judged to have defective oxidation of pyruvate, we carry out skin biopsies for fibroblast culture and usually initiate therapy with a diet high in fat and low in carbohydrate while the culture is being established. Once the fibroblasts are available, they are assayed for defects in the pyruvate dehydrogenase complex (PDHC) and in its first enzyme, pyruvate decarboxylase (E1). E1 has an α- and a β-subunit. Defects in E1a can be tested for by mutational analysis. The E2 transacetylase protein can be tested for by Western blot analysis, and some mutations have been defined. In patients with defects in this PDHC system, it is also useful to measure the activity of α-ketoglutarate dehydrogenase and lipoamide dehydrogenase (E3).

Muscle biopsy with histology and studies of electromyography and nerve conduction are employed to elucidate patients with myopathy, or abnormalities in mitochondrial structure. Ragged red fibers are frequently seen in disorders of mitochondrial DNA. Muscle may be used as a source for analysis of mutation in the DNA. There are patients in whom analysis of muscle reveals the heteroplasmy, while the blood does not. Fresh muscle obtained by open biopsy permits the best assessment of the activity of the complexes of the electron transport chain (Figures 47.5 and 47.6). In some laboratories, these assays are done on frozen muscle or on freshly isolated platelets. In addition, patients have been reported [13] in whom defective activities of the electron transport chain has been documented by assay of biopsied liver, even in patients in whom there have been no hepatic mechanisms of disease.

The lactate to pyruvate ratio in the blood is usually elevated in electron transport abnormalities, and this abnormality may trigger a muscle biopsy (see Figure 47.3). Ragged red fibers suggest analysis of mitochondrial DNA, while their absence suggests assay for a nuclear encoded defect in the respiratory chain. These complexes I to V are a mixture of mitochondrial and nuclear encoded proteins.

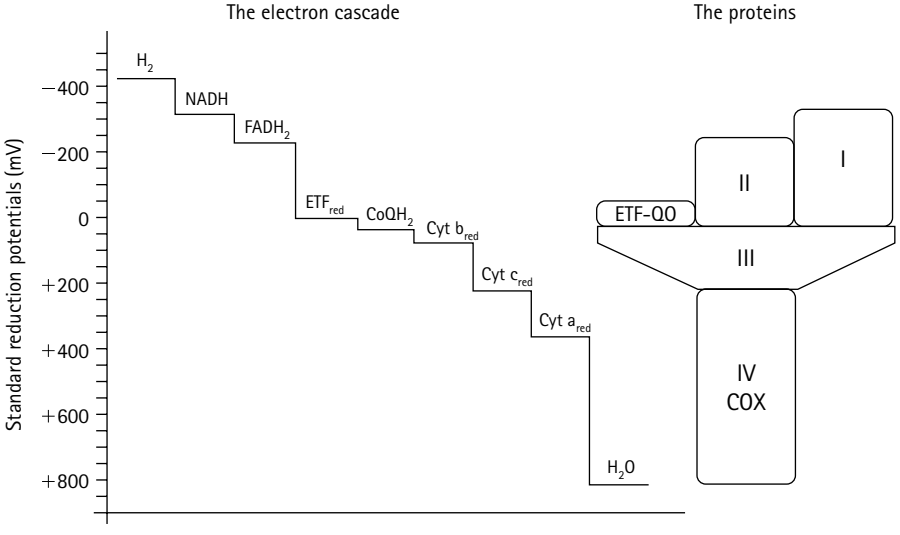

Figure 47.5 The mitochondrial electron transport chain. Lactic acidemia and an elevated lactate: pyruvate ratio may occur with any defect that interferes with the utilization of NADH. CoQ10, coenzyme Q10; Cyt, cytochrome; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD ($FADH_2$ its flavoprotein).

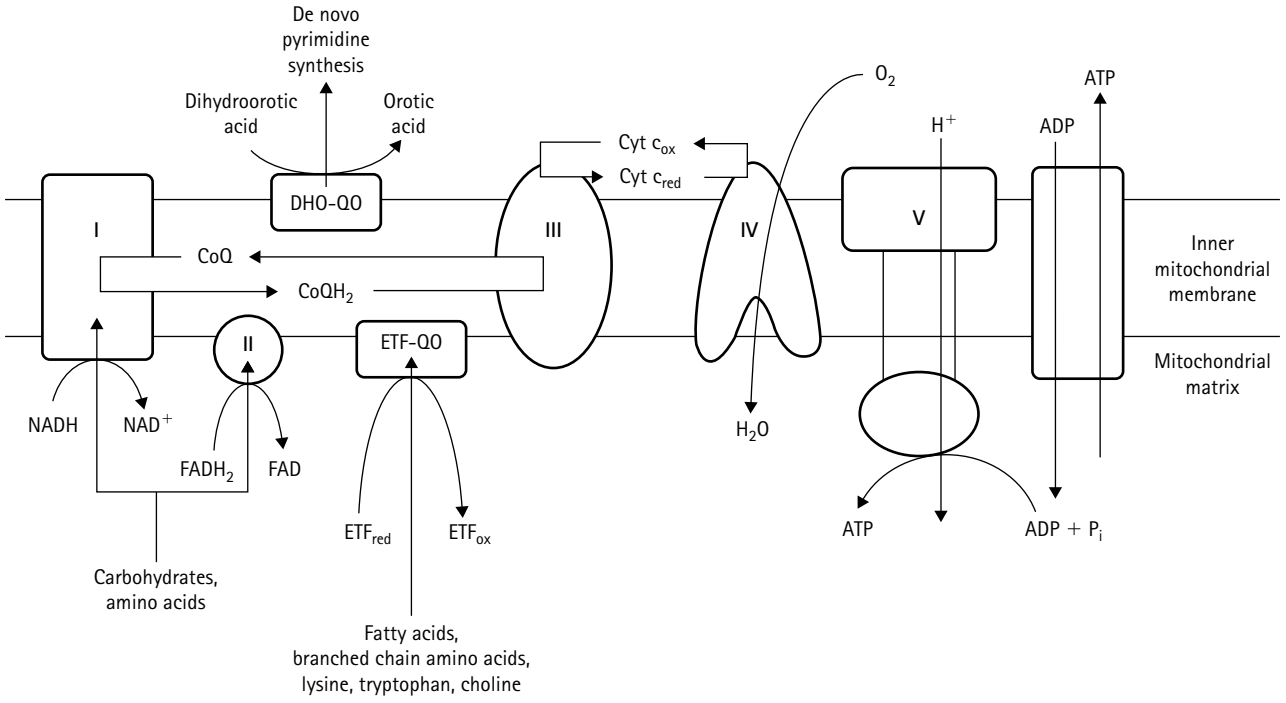

Figure 47.6 The electron transport chain.

Energy conversion takes place in mitochondria in which the exergonic oxidation/reduction reactions of the electron transport chain, as in chloroplasts and bacteria, are coupled to the endergonic synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate [14]. The electron flow generates a proton motive force. The ATP synthase is a large asymmetric enzyme complex of an F_0F_1 structure, in which the F_0 is a hydrophobic, membrane-embedded unit that serves as a proton channel, while the F_1 contains the nucleotide binding sites and catalytic sites for ATP synthesis. When solubilized and uncoupled from its F_0 energy source, the F_1 is capable of ATP hydrolysis, and this is why it is referred to as an ATPase.

The oxidative phosphorylation system is embedded in the lipid bilayer of the mitochondrial inner membrane. In addition to the five multiprotein enzyme complexes there are two electron carriers – coenzyme Q and cytochrome C. The ATP generated by oxidative phosphorylation may be used in the mitochondrion or transported out by the adenine nucleotide transporter for other cellular purposes. Each of the complexes of the electron transport chain except complex II contains proteins encoded by the mitochondrial DNA, as well as proteins encoded by nuclear DNA (Table 47.1). Mitochondrial DNA and its mutations are maternally inherited; nuclear DNA mutations in this system are inherited autosomally.

In addition to mutations in the genes coding for proteins of the electron transport chain, there are mutations causing mitochondrial disease in proteins involved in the assembly and maintenance of mitochondrial proteins. Increasingly, mutations are being found in the nuclear encoded functions of oxidative phosphorylation. A majority, so far, have been in complex I [15–19]. Some of these mutations, which disrupt respiratory chain function, do not produce lactic acidemia. Knowledge of the molecular mutation in a family permits accurate prenatal diagnosis, whereas biochemical methods have frequently been in error [20]. Among mutations in genes for proteins involved in the assembly of respiratory chain proteins SURF1, which functions in

Table 47.1 The genetic determination of the proteins of the electron transport chain

Complex	Name	Mitochondrial genes	Nuclear genes	Total
I	NADH: ubiquinone oxidoreductase	7	>44	>51
II	Succinate: ubiquinone oxidoreductase	0	44	44
III	Cytochrome bc	1	>10	>11
IV	Cytochrome c oxidase	3	10	13
V	ATP Synthase	2	14	16
Total		13	>82	>95

assembly of complex IV, is probably the best studied [21–23] and produces a severe clinical Leigh syndrome.

Molecular chaperones are required for the assembly of the catalytic F_1 component of the mitochondrial ATP synthase, and probably for many other proteins involved in mitochondrial function. Those for F_1 have been well studied in yeast and mutations in *Saccharomyces* are known. The human genes for orthologs are known. Their study may reveal novel mechanisms of mitochondrial disease.

Mitochondrial proteins synthesized in the cytosol must be transported into the mitochondria. Defects in the transport proteins could provide another novel mechanism of the pathogenesis of mitochondrial disease. Two of these protein complexes, translocation outer mitochondrial membrane (TOMM) and translocation inner mitochondrial membrane (TIMM), have been extensively studied in yeast in which the genes have been characterized. The human gene encoding an ortholog of TOMM 20 has been identified [24], and the human genome project has made the genes for other orthologs available. A search for abnormalities causing human disease is under way. Among the transporters of the mitochondria is an outer membrane transporter known as voltage-dependent ion channel (VDAC) and also known as mitochondrial porin, because it forms a pore, opening the membrane for anions like phosphate, chloride, and adenine nucleotides at low transmembrane voltage; at high voltage, it forms a channel for cations and uncharged molecules. A deficiency in VDAC has been reported in Western blot studies [25] in a patient with impaired myopathy and impaired oxidation of pyruvate in mitochondria of muscle. Lactate was elevated only mildly after 2 g/kg of glucose.

Concentrations of L-lactic acid in body fluids are a function of the concentrations of pyruvate and the ratio of NAD+ to NADH. This may be expressed as:

$$K = \frac{\text{Lactate}\,(\text{NAD}+)}{(\text{Pyruvate})(\text{NADH})(\text{H}+)}$$

When NAD/NADH ratios are constant, changes in lactate concentration are a function only of the metabolism of pyruvate, but the ratio of NAD to NADH reflects the oxidative state of the cell [26]. Hypoxia increases NADH, while oxidation regenerates NAD. The normal ratio of NADH to NAD in aerobic tissues is about 10:1, and the ratio of lactate to pyruvate does not normally exceed 15 [27]. Elevation of the cytosolic ratio of lactate to pyruvate, often considered to be above 25, indicates a disorder of oxidative phosphorylation. An elevation of the lactate to pyruvate ratio in the absence of elevation of the 3-hydroxybutyrate to acetoacetate ratio indicates a severe deficiency of pyruvate carboxylase (Chapter 48). The ratio of 3-hydroxybutyrate to acetoacetate may more closely reflect the redox state of the mitochondria. Conditions in which there is an excess of NADH will cause an elevated lactate to pyruvate or 3-hydroxybutyrate to acetoacetate ratio. When the electron transport chain is not functioning well, NADH cannot be converted to NAD and hence ATP is not produced. Compensation by conversion of pyruvate to lactate regenerates some NAD.

NADH is the fuel of the cytochrome chain (Figures 47.5 and 47.6). Therefore, an abnormality in a cytochrome such as cytochrome coxidase deficiency [28–30] will lead to abnormalities in these ratios because of diminished utilization of NADH. Defects in the respiratory chain may be demonstrated in cultured fibroblasts, as well as in muscle [28, 29, 31–39], but a group of patients has been described [34–38] in which defects in the respiratory chain in muscle were not demonstrable in fibroblasts. In a patient with fatal neonatal lactic acidosis [39], the ratio of lactate to pyruvate was 136:1. The ratio of 3-hydroxybutyrate to acetoacetate was 42:1. In fibroblasts, the conversion of 1-^{14}C-pyruvate and glutamate to $^{14}CO_2$ was defective, and when the cells were incubated with glucose, an elevated lactate to pyruvate ratio of 72:1 was observed. In control cells, the ratio was 20:1. The ratio has also been used to indicate a defect in pyruvate dehydrogenase where it is expected to be low or normal. It has been established that in classifying patients as having a respiratory chain dysfunction versus pyruvate dehydrogenase, the ratio of lactate to pyruvate is only useful when the level of lactate is high [40]. The ratio in the CSF may be used, as well as that of the blood.

We regularly employ a modified oral glucose tolerance test in which the standard 1.75 g/kg is monitored by assessment of concentrations of lactate, pyruvate, and alanine which may rise as much as four-fold over control levels in a patient with PDHC deficiency. This evidence of glucose intolerance may be useful in designing therapy that avoids carbohydrate and substitutes fat, as well in monitoring the efficacy of therapeutic interventions. Fructose loading has been used in assessing *in vivo* pyruvate dehydrogenase activity and its activation. After a 12–24-hour fast, blood samples are drawn for lactate, pyruvate, glucose, and insulin before and 45 minutes after an oral load of 1 g/kg of fructose. The test is then repeated after an oral glucose load. The rise in blood pyruvate and lactate was reported to be almost twice as great in the fasted as in the postglucose state, suggesting the conversion of pyruvate dehydrogenase to its active form by glucose feeding. Studies have not been reported on actual patients with problems with pyruvate dehydrogenase, and we have not found this to be especially useful. Empirically, an occasional patient has responded to fructose with a marked increase in lactic acid but, as a group, the patients with mitochondrial disease have not been reliably distinguishable from control in their response to fructose.

A postprandial rise in lactate (greater than two-fold) occurs in pyruvate dehydrogenase deficiency and also in glycogen storage diseases types 0, III, and VI/IX. In primary defects of the respiratory chain, the redox state may become more abnormal; in addition, there may even be a rise of total ketone bodies (paradoxical ketonemia). A postprandial fall of lactate occurs in glycogen storage

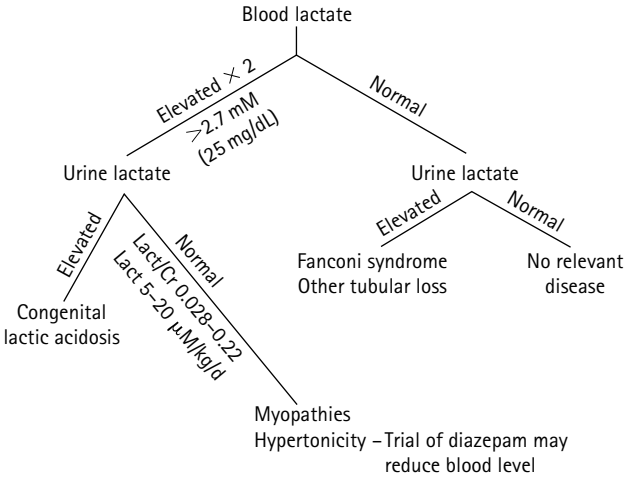

Figure 47.7 Assessment of lactate in urine as an aid to differential diagnosis of lactic acidemia.

disease type I and defects of gluconeogenesis. In glycogen synthase deficiency, concentrations of lactate and alanine are low when the patient is hypoglycemic, but feeding or a glucose tolerance test leads to elevated amounts of lactate, as well as hyperglycemia.

The urinary lactate may be useful in diagnosis [2] and the lactate to creatinine ratio has been employed for this purpose. The normal range is from 0.028 to 0.22. A schematic approach to the use of the blood and urinary lactate in differential diagnosis is shown in Figure 47.7. In congenital lactic acidosis, both the blood and urinary lactate should be elevated. However, each may be quite variable in any individual patient. Therefore, it is prudent in a patient suspected of having lactic acidosis to carry out a number of assays at various times.

CLINICAL ABNORMALITIES

The clinical manifestations of the congenital lactic acidemias have many similarities regardless of the specific causes. There are a number of distinct clinical syndromes [41, 42]. One of them is acute metabolic acidosis, usually neonatal or infantile. Congenital lactic acidosis was first described by Hartman and colleagues in 1962 [43] in patients with this clinical picture. A number of patients have been reported [44, 45] in whom the picture is that of recurrent episodes of acidosis with hyperventilation, any one of which may lead to coma and death. Attacks of unexplained vomiting may herald onset. Some patients have acute symptomatic hypoglycemia. This may lead to convulsive seizures, but seizures may also occur in the absence of hypoglycemia. Any patient with chronic lactic acidosis may develop pulmonary hypertension and this may be the cause of death.

Another presentation, especially in patients in later infancy or childhood, is with ataxia. This may be episodic or chronic. Episodes may be precipitated by stress, such as an intercurrent infection. Between attacks, the patient may be clumsy. Often, there is associated episodic neurologic degeneration.

Another type of presentation is with Leigh syndrome, or subacute necrotizing encephalomyelopathy [46–48]. This was essentially a histopathologic diagnosis, usually made at autopsy in an ultimately fatal disease. The neuropathologic picture resembles Wernicke encephalopathy in the basal ganglia, brainstem, and cerebellum, but in contradistinction to the picture in Wernicke disease, the mammillary bodies are usually spared. Spongiform degeneration is seen, as are increased vascularity and glial proliferation. Computed tomography (CT) or magnetic resonance imaging (MRI) (Figure 47.8) scans now provide the neuroimaging counterpart of the histology with hypodensity in the caudate and putamen (Figure 47.8) [48, 49]. Ultimately, the patient develops spasticity often with Babinski signs. Seizures occur in about one third of such patients. Blindness may supervene. There may be retinal pigment epithelium changes. Late in the course, deep tendon reflexes may be absent. Tracheostomy and artificial ventilation may be required. This picture of Leigh encephalomyelopathy is clearly independent of etiology. It has been described with deficiency of the PDHC [50] and in a patient with defective activation of the PDHC because of deficiency of pyruvate dehydrogenase phosphatase [47]. It may be seen in patients with NARP mutation.

Hepatic presentations, particularly hepatic failure in the neonatal period, have been recognized as a presentation of disorders of oxidative phosphorylation [51]. In a series of 22

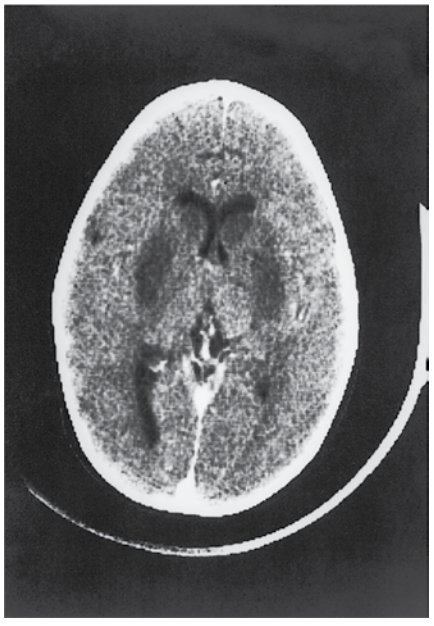

Figure 47.8 CT scan of the head of a patient with lactic acidemia and Leigh encephalopathy, illustrating advanced degenerative changes in the brain stem, as well as the basal ganglia.

patients; nine had a severe neonatal presentation in which hepatic failure led usually to a rapidly fatal course. They also had hypotonia, psychomotor retardation, seizures, and hypoglycemia. A somewhat delayed onset from 2–18 months was often fatal.

We have studied a small subgroup of patients with severe deficiency of the PDHC in whom there was a recognizable syndrome of dysmorphic features (Chapter 56). A sibling had a similar clinical appearance. Our first patient appeared to be cortically blind in infancy. Another patient had gross abnormalities in the morphogenesis of the brain.

Another group of patients has the picture of a metabolic myopathy or ophthalmoplegia [50–54]. In some, there was associated neurodeafness or early cataracts. Two siblings had sideroblastic anemia. Many of these patients have had muscle weakness, usually of insidious onset, in a pattern of a proximal muscular dystrophy. Ptosis, facial muscle weakness, and cardiomyopathy have also been seen. Electron microscopy may reveal large mitochondria, often with a bizarre appearance. Some patients have ragged, red fiber changes in the histology of muscle. Many of these have had abnormalities in mitochondrial DNA.

Deafness resulting from sensitivity to gentamicin has been observed in patients with the A1555G mutation in mitochondrial 12S ribosomal RNA [55]. One patient was frequently treated with gentamicin because of febrile episodes associated with neutropenia induced by chemotherapy for leukemia. It has been recommended that the mutation be sought in patients frequently treated with aminoglycosides.

Lactic acidemia has been reported [56] as a consequence of thiamine deficiency is a patient with malignancy receiving total parenteral nutrition in which there was no thiamine. Treatment with IV thiamine led to a normal concentration of lactic acid.

Mutations in mitochondrial genes, encoded by the nuclear and mitochondrial DNA lead to inadequate production of energy of oxidative phosphorylation. tRNA methyltransferase (*TRMT5)* catalyzes the methylation of guanosine at position m'G37. Mutations in *TRMT5* were found [57] in a man with exercise intolerance, exertional dyspnea, and lactic acidemia, as well as exocrine pancreatic in failure, cirrhosis, and renal glycoside. An unrelated male had delayed psychomotor development, cardiomyopathy, and lactic acidemia. Each had the same frameshift variant along with missense variant p.Arg291His and p.Met386Val.

A recent addition to mitochondrial diseases has been a syndrome associated with LONP1, which evokes mitochondrial AAA* Lon Protease [58]. Entitled CODAS syndrome, cerebral, ocular, dental, auricular, skeletal syndrome (MIM600373), the disease appears to result from alteration rather than complete absence of a complicated function of LON-dependent proteins, in which there is defective ATP-dependent proteolysis, swollen mitochondria, defective cytochrome oxidase, and impaired mitochondrial function. Patients have flattened midface, ptosis, crumpled ears, paretic vocal cords, and nuclear cataracts.

Primary deficiency in the activity of CoQ10 has been described in five patients with mutations in CoQ4 [59]. Four patients had prenatal or neonatal onset of disease and died within hours of birth. The fifth had a more indolent disease and was 18 at report. Slowly progressive neurodegeneration began at 10 months. He learned to walk with a spastic gait at three years, but he lost ambulation by six years and began having seizures at 12 years. All had impressive lactic acidemia. CoQ10 activity was reduced in muscle and fibroblasts. Loss of function of CoQ4 was virtually complete. Mutations included c.433C>G (p.Arg145Gly) and c.190C>T (p.Pro645Ser). A high-energy requirement for cardiomyocytes is consistent with the frequency of cardiomyopathy in inherited mitochondrial disorders.

PATHOGENESIS

Patients with evidence of defective activity of many mitochondrial enzymes exemplify the complex nature of the pathogenesis of mitochondrial disease. For instance, mitochondrial DNA depletion (Chapter 56) leads to defective activity of most of the complexes of the electron transport chain. Similarly, patients were reported [60] in whom there was defective activity of pyruvate and 2-oxoglutarate dehydrogenase complexes, NADH cytochrome c reductase, succinate dehydrogenase, and succinate cytochrome reductase. This fatal disease in three siblings was shown by microcell-mediated transfer to a panel of mouse-human hybrids to be under control of a nuclear gene on chromosome 2 at 2p13-14.

Metabolic coupling has been observed in the relationship between glial cells and neurons involving the metabolism of lactate [61]. Astrocytes appear to be the main site of glucose uptake during neuronal activity. Glucose is then processed glycolytically in these astrocytes, which then release lactate which is the metabolic substrate used by neurons.

TREATMENT

Acute treatment of metabolic acidosis may require large amounts of sodium bicarbonate. Sodium citrate may be ineffective in a patient with an oxidative defect because citric acid cycle function is impaired. Chronic treatment may be undertaken in patients with lactic acidemia prior to the establishment of a definitive diagnosis, provided enough is known about the underlying pathophysiology. Thus, patients with disorders of gluconeogenesis should avoid fasting and require IV glucose during intercurrent illnesses in which the oral route is not available, as in the vomiting patient. A diet high in carbohydrate is therapeutic and cornstarch supplementation may be helpful. Management of mitochondrial disease has been the subject of a consensus statement [62].

Patients with disorders of oxidation are, in contrast, often glucose-sensitive and respond with reduction in lactate

concentrations to a diet high in fat [63]. Diets employed contain 50 percent or more of the calories from fat. They do not have to be ketogenic.

Lactate levels can be lowered in some patients by the administration of dichloroacetate (DCA), regardless of the cause [64–68]. It is not generally recommended in disorders of gluconeogenesis, because DCA can itself produce hypoglycemia. Its use has been employed experimentally in a variety of other lactic acidemic conditions. Dichloroacetate activates the PDHC by inhibiting PDH kinase. *In vivo,* this compound reduces concentrations of lactate, pyruvate, and alanine, and increases the percentage of the active form of PDHC in brain, liver, and muscle. It has been used to treat congenital lactic acidosis [67–69], and levels of lactic acid have been improved. Neurologic improvement has been elusive in most patients reported, but there have been some successes. Peripheral neuropathy can be expected to worsen with DCA, and some patients have developed peripheral neuropathy [69].

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48

Pyruvate carboxylase deficiency

MAJOR PHENOTYPIC EXPRESSION

There are three phenotypes in each of which concentrations of lactic acid and alanine are elevated and activity of pyruvate carboxylase is deficient.

i. In the complex type first described from France: severe lactic acidemia, usually fatal in the early months of life, hyperammonemia, citrullinemia, and hyperlysinemia.
ii. In the simple type common in American-Indians: delayed development and infantile episodes of metabolic acidosis with lactic acidemia.
iii. In a more benign presentation, episodic acidosis only.

INTRODUCTION

Pyruvate carboxylase (EC 6.4.1.1) is a biotin-containing mitochondrial enzyme, which catalyzes the conversion of pyruvate to oxalacetate by CO_2 fixation (Figure 48.1) [1, 2]. As in the case of other carboxylases, the reaction mechanism is a two-step process in which biotin is first carboxylated and then the carboxyl group is transferred to the acceptor, pyruvate [3, 4]. There is a separate catalytic site for each of the two steps. The enzyme is a tetramer of 500 kDa whose individual equal-sized protomers have a different structure from other biotin-containing carboxylases [5], but the highly conserved amino acid sequence at the biotin site of biotin-containing carboxylases, Ala-Met-Lys-Met is present in pyruvate carboxylase [6]. The biotin is linked to the ε amino group of the lysine.

Pyruvate carboxylase is an important regulatory enzyme with highly tissue-specific roles. In liver and kidney, where its activity is highest, it catalyzes the first step in gluconeogenesis from pyruvate in which the oxaloacetate formed is converted via phosphoenolpyruvate carboxykinase (PEPCK) to phosphoenolpyruvate, and ultimately to glucose and glycogen. It is regulated via acetylCoA, an allosteric activator, and the stimulant

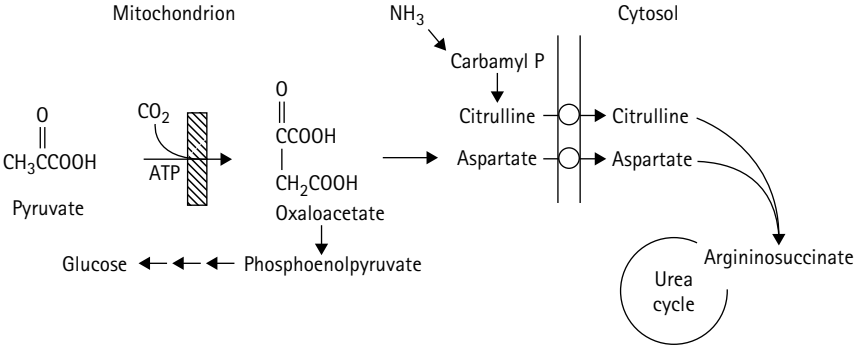

Figure 48.1 The pyruvate carboxylase reaction and the metabolic consequences of its deficiency.

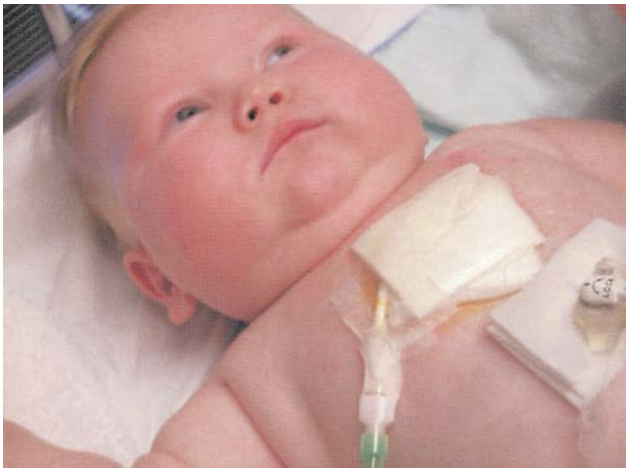

Figure 48.2 JM: A five-month-old infant with pyruvate carboxylase deficiency presented first in coma. He was hypotonic to flaccid and had inverted nipples. Respirations were Kussmaul. Metabolic imbalance was judged incompatible with life, but liver transplantation reversed many features of the disease.

ratio of adenosine triphosphate/adenosine diphosphate (ATP/ADP). Conditions under which acetyl groups are generated stimulate gluconeogenesis at this step [7]. In lipogeneic tissues, such as adipose and adrenal, the enzyme participates in the synthesis of acetyl groups and reducing groups for transport into the cytosol. In other tissues, such as brain, muscle, and fibroblasts, it has an anapleurotic role in the formation of oxaloacetate and the maintenance of four carbon intermediates for the citric acid cycle [8]. Anapleurotic is from the Greek verb to fill up. Experience with liver transplantation in this disease (Figure 48.2) [9] indicates that the liver can take over this citric acid cycle-related function for the entire body. In brain, the enzyme is active not in neurons but in astrocytes, where it is involved in the synthesis and supply for the neurons of glutamine, a major precursor of the glutamate and 4-aminobutyrate neurotransmitters [10].

Deficiency of pyruvate carboxylase was first described in patients with Leigh syndrome [11–14]. It is now thought that this was a function of instability of this enzyme, especially in material obtained at autopsy. In assays of biopsied liver in six patients with Leigh syndrome and of cultured fibroblasts in five, pyruvate carboxylase was not deficient [15].

The cDNA for pyruvate carboxylase has been cloned and sequenced and localized to chromosome 11 at q13.4-13.5 [6, 16–19]. Absence of mRNA for the enzyme was found in four of six patients with the fatal infantile disease who also lacked pyruvate carboxylase protein [20]. Mutations have been found in both the type A or simple form, as well as the type B severe infantile form [21], none of which patients had any enzyme activities. In a number of them, frameshifts led to premature terminations.

CLINICAL ABNORMALITIES

Complex, French, or European form

In the complex form, severe neonatal lactic acidosis is the presenting feature [22, 23]. The initial acidosis may be fatal and many patients have died by three months of age. Most have hepatomegaly. Metabolic acidosis may lead to dehydration, coma, shock, and apnea. This disorder has now been observed in North American, Egyptian, and Saudi Arabian patients [9, 20, 24–26].

The term 'complex' refers to the biochemical findings in this group of patients in whom the occurrence of hyperammonemia and citrullinemia is characteristic [23, 25, 27]. Hyperlysinemia is also seen, as it is in other hyperammonemic conditions levels of proline may also be elevated. Hypoglycemia may occur, but it is usually not a major problem. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) may be elevated. Concentrations of alanine and proline are high, and there are elevated amounts of 2-oxoglutarate in the urine. Levels of lactate may be very high, and levels of pyruvate are elevated. Abnormal redox balance in which the cytosol is more reducing is indicated by a high ratio of lactate to pyruvate in the blood, while a more oxidizing mitochondrial environment is indicated by a high acetoacetate to 3-hydroxybutyrate ratio [9]. The major metabolic abnormality, significant of citric acid cycle aberration, is the massive ketoacidosis and ketonuria.

In nine patients reported [28], the largest series to date, neurologic features were emphasized. These include axial hypotonia, hypokinesia and slowness of movements a disorder characterized by high amplitude movements of the limbs, usually all of our [28]. Magnetic resonance imaging (MRI) showed high-signal white matter and periventricular cysts. All had lactic acidemia and tachypnea, elevated transaminases, enlarged liver, and in some coagulopathy and hepatic failure. Dysmorphic features included epicanthus, long filtrum, and thin upper lips [27].

Simple or American–Indian form

In the American-Indian form, there may be episodes of acute metabolic acidosis with lactic acidemia in the first six months of life, or the first evidence of abnormality may be slowness of development [29–31]. By the first year, most have impaired mental development and many have failure to thrive, vomiting, or irritability. This clinical picture has been seen frequently in Saudi Arabia (Figures 48.3–48.8), as well as among American Indians [30]. It has been encountered in North American Caucasians and in Japanese [32]. Macrocephaly has been observed, as well as subdural effusions (Figures 48.4 and 48.5). These effusions have also been encountered in the complex phenotype [9]. Impaired mental development may be severe (Figures 48.5–48.8)

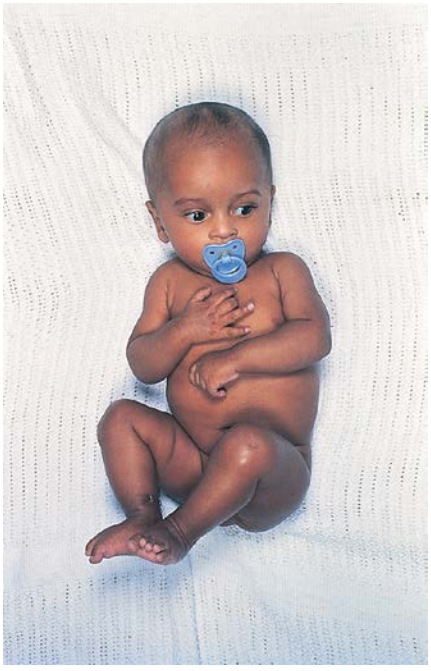

Figure 48.3 TYAAH: A six-month-old Saudi male infant with pyruvate carboxylase deficiency. He had recurrent episodes of lactic acidosis. Plasma ammonia was normal. Levels of alanine were elevated and proline was moderately elevated. Pyruvate carboxylase in fibroblasts was 11 percent of control.

Figure 48.4 TYAAH: The head was relatively large, just above 2 SD above the mean, while length and weight were below the 50th percentile. An electroencephalogram (EEG) showed diffuse slowing consistent with metabolic encephalopathy, and later he developed seizures. Computed tomography scans revealed a subdural effusion. By two years of age, he had spasticity and was appreciably developmentally delayed.

Figure 48.5 OH: A six-month-old female infant with pyruvate carboxylase deficiency. She presented at 2 days of age with grand mal seizures. The tonic neck posture is normal at this age, but she also had choreoathetosis. She had lactic acidosis and undetectable activity of pyruvate carboxylase.

[29]. Hypoglycemia occurred on fasting for 24 hours at which time the concentration of glucose was 1.8 mmol/L and lactate was 6.2 mmol/L, while the 3-hydroxybutyrate was greater than 2 mmol/L. A number of these patients have been ketotic at times of acute acidosis. There was no increase in blood glucose after an alanine load.

Seizures are frequently seen (Figure 48.5). One patient [29] presented at three months with fever and mild generalized seizures. Between the ages of five and nine months, he was admitted to hospital three times for failure to thrive and developmental delay; on each occasion, mild metabolic acidosis was noted, but not evaluated further.

Figure 48.6 OH: A previous sibling had died of intractable lactic acidosis.

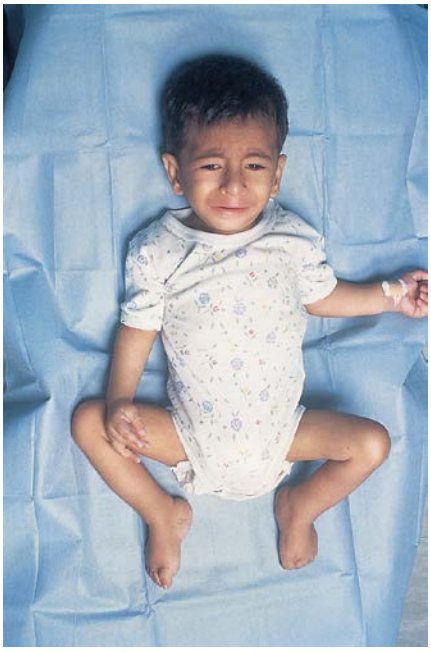

Figure 48.7 MSG: A 16-month-old Saudi male with pyruvate carboxylase deficiency. Tachypnea and acidosis were noted soon after birth. Initial pH was 7.00 and the serum bicarbonate was 5 mEq/L. Lactate was 6.2 mmol/L and alanine 1074 μmol/L; proline was 775 μmol/L. The activity of pyruvate carboxylase in fibroblasts was 4 percent of the control mean.

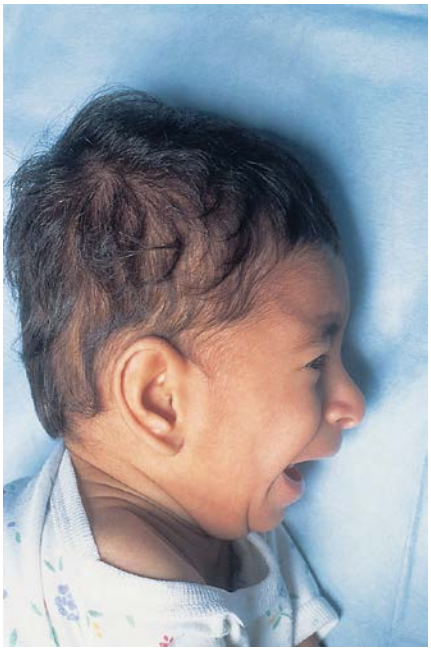

Figure 48.8 MSG: He had microagnathia and hypospadias. By 16 weeks, he could not sit unassisted or roll over. At 12 months, his head circumference was at the 50th percentile for four months, but length was at the same level and weight even further behind. Computed tomography scan revealed hypodense periventricular white matter.

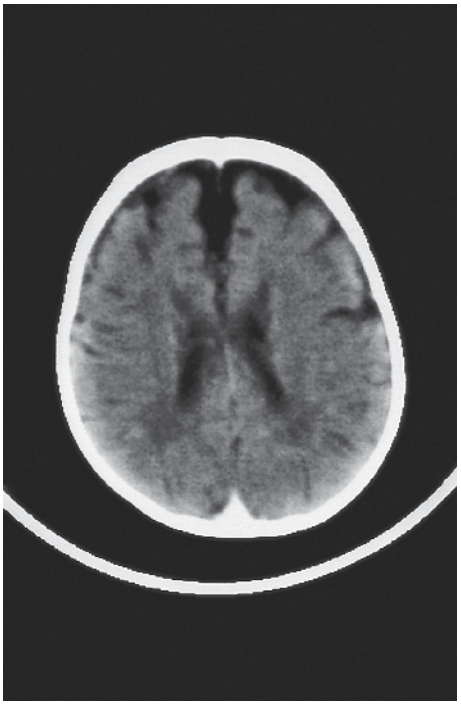

Figure 48.9 Computed tomography scan of the brain of the five-month-old patient shown in Figures 48.5 and 48.6. There was already marked atrophy of the brain, particularly evident over the frontal lobes. She died shortly after discharge.

At nine months, he had a severe metabolic acidosis and was diagnosed as having lactic acidemia. The level was 6.5 mmol/L. By 46 months, he was microencephalic and had severely impaired mental development; he was unable to sit or feed himself, and he was not interested in his surroundings. He had numerous sudden episodes of lactic acidosis with tachypnea and a blotchy cyanosis of the extremities. He died at that age of pneumonia and severe acidosis. This patient and others in this group had proximal renal tubular acidosis. Renal tubular acidosis has also been encountered in the complex phenotype [9].

Electroencephalography (EEG) may show prominent theta waves and abnormal slow wave activity. Histologic examination of the brain has shown depletion of neurons and poor myelin formation, as well as increased ventricular size. These changes are evident in neuroimaging (Figure 48.9). Similar findings are seen in the complex form, in which there may also be cavitated infarcts or cortical cysts [22, 26]. Histologic examination of the liver in the simple and complex forms reveals steatosis.

Third form of pyruvate carboxylase deficiency

The third form of pyruvate carboxylase deficiency was described in a single patient [33], who had frequent episodic lactic acidosis in infancy. She was otherwise well and developed normally. By seven years of age, she had slight dysarthria and learning problems in mathematics. There

was no failure to thrive; she was over the 95th percentile for height and weight.

Another patient reported as atypical [34], largely because of long survival, presented at 3 days of life with ketoacidosis, tachypnea, and hypotonia. By nine years, he had mild global developmental delay. Another unusual feature was MRI evidence of high-signal intensities in the subcortical white matter of the frontal temporal area of the brain.

Molecular evidence of mutational heterogeneity may ultimately recognize the three phenotypic distinctions as impractical. The metabolic abnormalities found in the complex phenotype are distinctive.

GENETICS AND PATHOGENESIS

All forms of pyruvate carboxylase deficiency appear to be inherited in an autosomal recessive fashion. A founder effect has been postulated for the abnormal gene in the Canadian Indians, all of whom speak the Algonquin language [35].

Enzyme activity can be measured in lymphocytes and cultured fibroblasts [20, 31], as well as tissues. Levels of enyzme activity have been very low, less than 5 percent of control, regardless of phenotype, but some activity is often measurable even in the most severely affected patients [9]. Assessment of the presence of pyruvate carboxylase enzyme protein has revealed differences. The enzyme can be labeled with ^{3}H-biotin or ^{35}S-streptavidin prior to sodium dodecyl sulfate (SDS) gel electropheresis, which reveals a normal 125 kDa band in the two milder groups of patients, while no band was detected in the fatal severe neonatal form [20, 32]. Immunoprecipitation of ^{35}S-methionine-labeled protein with antibody to normal enzyme indicated absence of the protein in a number, but not all, of the patients with this form of the disease. Absence of mRNA for the enzyme was found in four of six patients with this form of the disease when tested by Northern blot assay with a cloned cDNA probe [20].

The cDNA for pyruvate carboxylase codes for 19 exons over 16 kb [36]. In studies of the rat gene, alternative tissue-specific transcripts led to greater expression in liver and kidney than in other tissues [37]. Among the Amerindian patients, homozygosity for G1828A which changed alanine 610 to threonine, was found in the Ojibwa or Cree, consistent with a founder effect. Carrier rates were as high as one in 10. In another group of Amerindians, there was a C2229T change, converting methionine 743 to isoleucine.

In five unrelated patients with the severe infantile phenotype, there was at least one truncating mutation [21]. In two male siblings with this phenotype, there was compound heterozygosity for two deletions that were predicted to lead to frameshift, premature termination, and nonsense-mediated mRNA decay [38].

In a report of eight novel mutations in seven patients [39]. all had the severe phenotype and had severely decreased enzyme activity. Four had bilateral cystic changes on cerebral imaging. It had previously been thought that patients with at least one missense mutation had the milder clinical features of the disease, but two patients indistinguishable clinically from the other patients in this series homozygous missense mutations c.615G>C(p. Arg205ser) in exon 4 and c.2606G>A (p.Gly869Asp).

Heterozygote detection by assay of enzyme activity is sometimes possible in a family, but the range of normal is so great that a normal result may be inaccurate [37, 40]. At the other extreme, an apparent homozygote for the third type of disease with severe chronic lactic acidosis and no other abnormalities displayed 50 percent of control activity [41, 42]. If the mutation is known, carrier deletion is simplified.

Prenatal diagnosis has been accomplished in families at risk [43–45]. In one family in which a sibling had died of severe neonatal disease, biotin-labeled enzyme protein was absent in amniocytes.

The pathogenesis of lactic acidemia appears intuitively to be a direct consequence of the failure to metabolize pyruvate by this pathway. Pyruvate does not accumulate, but is rather converted to alanine and lactate. Hypoglycemia has been observed [37] in each of the forms of the disease, and appears likely to be a consequence of abnormal gluconeogenesis.

The complex biochemical picture reminiscent of a defect in the urea cycle appears to result from depletion of intracellular oxaloacetate and aspartate [22, 23, 27]. Aspartate is a source of the second nitrogen of urea (Figure 48.1); its deficiency would lead to citrullinemia and hyperammonemia. Aspartate is also involved in the shuttle of reducing equivalents from cytosol to mitochondria [46] by which the NAD+/NADH ratio (nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide) is very oxidized in the cytosol and reduced in mitochondria; its lack would make the cytosol more reduced and the mitochondria more oxidized, as occurs in this phenotype.

Abnormality in the anapleurotic formation of glutamine in astrocytes is consistent with diminished concentrations of glutamine found in autopsied brain of a three-year-old patient [47]. Glutamine concentrations were low in the plasma and cerebrospinal fluid (CSF) of five living patients. Lactic acid itself may be directly toxic to brain. Depletion of oxaloacetate and interruption of the citric acid cycle would be expected to affect adversely the energy metabolism of the brain.

Experience with liver transplantation [9] has permitted a dissection of pathogenetic features of the disease. Prior to transplantation ketoacidosis could be ameliorated by large amounts of intravenous glucose, but enteral glucose was much less effective and large amounts of enteral glucose markedly worsened the lactic acidosis. Orthotopic transplantation of the liver completely abolished life-threatening ketoacidosis and with it the systemic metabolic acidosis. Thus, it was clear that the acidosis and its enormous requirement for sodium bicarbonate to maintain neutrality is caused by the ketoacidosis, not by the lactic acidemia, because lactic acidemia, cerebral lactic acid elevation, and lactic aciduria persisted.

The provision of enzyme in the transplanted liver also abolished the abnormal redox state of this disease in which the cytosol is reducing with a high lactate to pyruvate ratio, while the mitochondrial environment is more oxidizing as indicated by a high ratio of acetoacetate to 3-hydroxybutyrate.

Glutamine levels were low and did not improve with liver transplantation. This could reflect a role for glutamine depletion in the cerebral manifestations of this disease. Cerebral depletion of glutamine could affect the replenishment of glutamate and 4-aminobutyrate (GABA) neurotransmitter pools. Liver transplantation ameliorated the lactic acidemia, but lactic acid concentration in the CSF fluid remained elevated post-transplantation, and the lactate to pyruvate ratio was unchanged. The central nervous system effects of the disease were not reversed, but there was surprising improvement of function during the first year of follow-up evaluation.

TREATMENT

Metabolic acidosis and renal tubular acidosis have been treated in most patients with sodium bicarbonate. In acute episodes, parenteral fluids are required. A trial of biotin would appear prudent in any patient, but to date no responses have been reported.

Supplementation with aspartic acid appears to be a rational approach to a shortage of oxalacetate [9, 29, 48]. Treatment appeared to reduce levels of lactate and alanine and the number of acidotic attacks [29], but in our patient, much larger amounts of Naaspartate, along with Nacitrate and Nasuccinate, failed to alter the life-threatening ketoacidosis. Glutamine 400–800 mg every four hours was thought to have diminished the number of acidotic episodes in a patient [29], but the disease proved relentless. Treatment with dichloroacetic acid is effective in ameliorating the lactic acidemia [9].

Hepatic transplantation abolished the renal tubular acidosis, as well as the ketoacidosis. None of the treatments reported have had a major effect on the cerebral features of the disease.

A small number of patients have been treated with triheptanoin in an approach to anaplerotic therapy [49, 50]. The treatment has been deemed unsuccessful with deaths at seven and eight months [50] and six months [49]. However, in one of these patients [49] hepatic failure was reversed following treatment, and there was transport of C5 ketone bodies access the blood-brain barrier and increased concentrations of glutamine and γ-aminobutyric acid (GABA) in the CSF.

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49

Fructose-1,6-diphosphatase deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoglycemia, lactic acidosis, impaired gluconeogenesis, and deficiency of hepatic fructose-1,6-diphosphatase.

INTRODUCTION

Deficiency of fructose-1,6-diphosphatase (FDP) (fructose-1,6-bisphosphatase) was first recognized in 1970 by Baker and Winegrad [1], in a girl with hypoglycemia and metabolic acidosis. A sibling had died of a similar illness. In subsequent reports in 1971 by Baerlocher and colleagues [2] and by Hulsmann and Fernandez [3], there were multiple affected siblings of consanguineous matings.

The enzyme FDP (EC 3.1.3.11) provides an essential step in the pathway of gluconeogenesis (Figure 49.1). The enzyme catalyzes the irreversible conversion of fructose-1,6-diphosphate to fructose-6-phosphate. Another enzyme, phosphofructokinase, and adenosine triphosphate (ATP) are required to take this reaction in the reverse direction. The enzyme is most active in liver and kidney; and the liver enzyme is highly regulated [4]. Deficiency has most often been documented in the biopsied liver. The gene (*FBP1*) has been cloned and localized to chromosome 9q22.2-22.3 [5]. Seven exons span 31 kb. The common mutation in Japanese people is an insertion, 960–961insG [6], which was also the most frequent mutation in a non-Japanese population [7, 8]. This mutation causes a frameshift and premature chain termination, as does 966del, and expression studies have shown both to be pathogenic. The disease is clearly genetically heterogeneous and a variety of other mutations has been found.

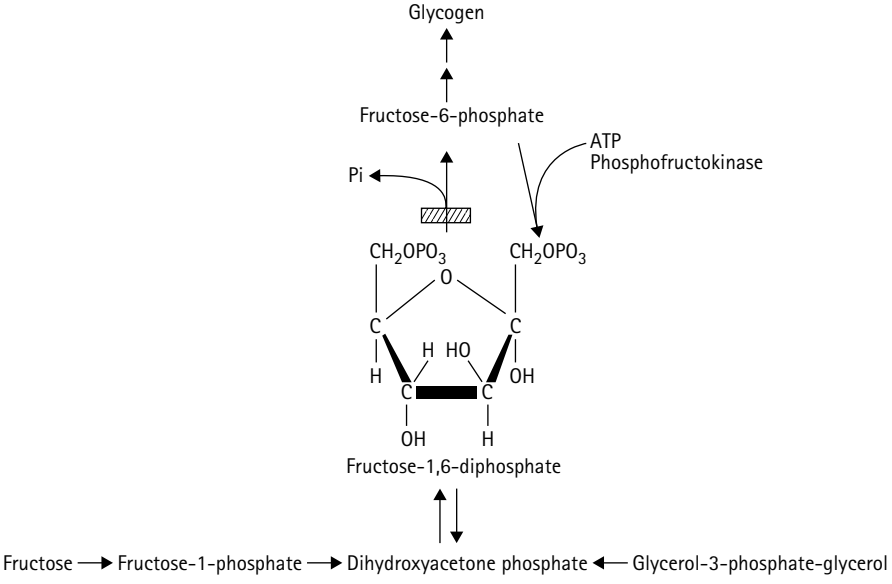

Figure 49.1 The fructose-1,6-diphosphatase reaction and its role in gluconeogenesis. The conversion of fructose-6-phosphate to fructose-1,6-diphosphate in glycolysis is catalyzed by another enzyme, 6-phosphofructose-1-kinase.

CLINICAL ABNORMALITIES

FDP deficiency is a cause of life-threatening metabolic acidosis in the neonatal period. A history of a previous sibling who died in acidosis has often been the alerting episode that led to early diagnosis and survival in the subsequent affected infant [1, 2]. Onset in about 50 percent of patients is between one and four days of age. Most of the patients present before six months. An exception first developed symptoms at four years [9].

The first symptom in the neonatal presentation is usually hyperventilation. There may be irritability, but progression is usually rapid to somnolence, coma, apnea, and cardiac arrest [2]. Physical examination may reveal tachycardia and hepatomegaly. Laboratory evaluation reveals hypoglycemia, severe acidosis, and lactic acidemia [10]. The episode usually responds well to vigorous therapy with parenteral fluids containing glucose and sodium bicarbonate.

Subsequent episodes usually follow fasting, usually precipitated by intercurrent infections. Onset may be with vomiting and anorexia; attendant fasting leads to hypoglycemia and metabolic acidosis. In one patient, episodes of hyperventilation began when the infant was weaned and baby foods were begun at six months [10]; on admission, she was hypoglycemic and the lactic acid concentration was 20 mmol/L. Patients have been described as ketotic and the urinary test for ketones is often positive during the acute episode, but the disease has been classified among hypoketotic causes of metabolic acidosis and coma [11]. In the absence of gluconeogenesis, ketones would be expected to accumulate as soon as hepatic glycogen is depleted, and the usual crisis is associated with ketosis. Vomiting is not a common response to fructose and patients do not have the aversion to fruit and its products, seen regularly in hereditary fructose intolerance. Nor do they develop proximal renal tubular dysfunction after fructose, as do those former patients.

Hepatomegaly develops regularly in infancy, but there are usually no signs of liver disease [11, 12]. Neonatal hyperbilirubinemia of a severity requiring exchange transfusion was reported in three infants [13]. Failure to thrive may be seen rarely.

There may be convulsions or other manifestations of hypoglycemia. There may be flushing [2], or pallor and sweating. Vomiting may be complicated by hematemesis [14, 15]. Hypotonia and muscle weakness have been observed. The electroencephalograph (EEG) may be abnormal during the acute attack and normal later. Fast spindle-shaped bursts on a slow amplitude pattern have been described [2], as well as a slow-wave pattern [16]. Intellectual development is usually normal (Figures 49.2 and 49.3). Of course, impaired mental development, as well as death may accompany neonatal or early infantile hypoglycemic crises, but fasting tolerance improves with age, and patients normal by childhood usually develop normally. In addition to the lactic acidemia, analysis of the blood reveals increased concentrations of alanine and uric acid [17]. In some attacks, there may be acidosis without hypoglycemia. Glycerol and glycerol-3-phosphate have been found in the urine [18, 19].

Figure 49.2 NM: An infant with fructose-1,6-diphosphatase deficiency. She did not have neonatal hypoglycemia. Her first episodes occurred following exposure to fruit juices in the infant's diet.

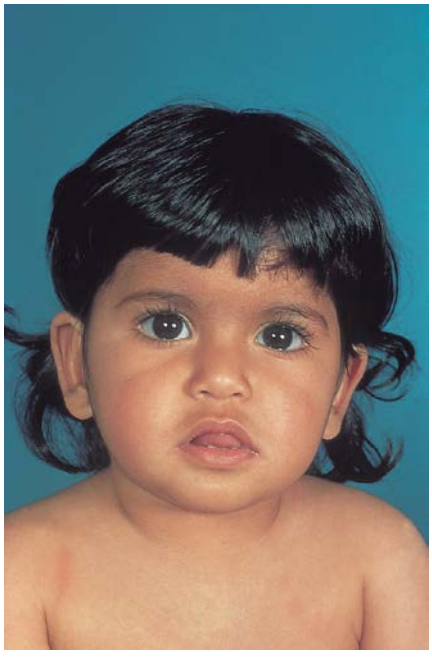

Figure 49.3 NM: Close up of the face, an appearance consistent with her normal intelligence.

GENETICS AND PATHOGENESIS

The disease is transmitted in an autosomal recessive fashion. There is no ethnic predominance. Consanguinity was observed early [2, 3]. Most reports have been of Europeans, but there have been reports from the United States, Japan, and Lebanon [10, 20–25], and among the European families in one series a majority were Turkish [25]. Among nine patients from six families in Israel, two families were Jewish, three Arabic, and one Druze [26]. The disease is common in Saudi Arabia, where 14 patients were reported [27]. Parents of patients have been documented to have intermediate levels of FDP activity in liver [14, 28]. Testing for heterozygosity by assay of the enzyme in leukocytes may be unreliable. Prenatal diagnosis and heterozygote detection can be accomplished if the mutation is known.

The defective enzyme in patients has usually been identified in the assay of biopsied liver [1–3, 29]; a majority of patients have little or no activity. Others have had 20 percent or less of control activity. The enzyme is also active in kidney [3, 15] and jejunum [30], and the defect has been identified in both these tissues. Assay of leukocytes is controversial and the activity in normal individuals is quite low. The enzyme is not expressed in fibroblasts or amniocytes. The defect has been documented in tissues obtained at autopsy, but the results must be interpreted with caution because of rapid inactivation of the enzyme with autolysis [31]. The documentation of FDP enzyme activity in muscle in patients in whom activity was deficient in liver and kidney indicates that the enzyme in muscle is coded by a different gene [10, 15]. Diagnosis has generally required the assay of the enzyme in biopsied liver, but mutational analysis may permit its avoidance. The enzyme in blood is expressed only in monocytes. Culture of monocytes in media rich in calcitriol has been reported [32] to permit the diagnosis by enzyme assay. A radiochemical method has developed that detects defective (absent) enzyme activity in isolated monocytes before and after incubation with calcitriol in tissue culture media [33].

The gene *FBP1* on chromosome 9 has seven exons. The insG960-961 accounted for 46 percent of 22 mutant alleles in Japanese and 14 of 28 mutant alleles in non-Japanese [7]. Its frequency in diverse populations suggests a propensity for mutation. Frameshift was also observed with 807delG and 704-705insC [7]. Point mutations included A177D, N213K, and G294V [7, 32]. Premature termination (Q30X) was homozygous in a Japanese patient [6]. In a small number of patients, a concerted search for mutation has failed to reveal any [6]; the possibility of mutation in a promoter region has been suggested. In a Japanese patient, C851G leading to p.F194S was found in compound with p.P284R [34]. In a Swedish patient, two heterozygous mutations were p.G620R and p.G294E, while another had p.Y216X and a large 300-kb deletion [33].

Deficient activity of FDP interferes with gluconeogenesis, making the patient dependent on exogenous sources of glucose. The fasting that normal infants often undergo in the first days of life makes for a neonatal presentation in patients with little residual enzyme activity [35]. If tested with a provocative fast, patients become hypoglycemic when stored glycogen is exhausted. This may occur in a few hours in an infant [15] or after 14–20 hours in an older patient [1, 2, 15, 34]. Depletion of glycogen previously synthesized from glucose by injection of glucagon early in the fast ensures that gluconeogenesis is being tested adequately (Chapter 49) [10]. The fed state response to glucagon may be normal. Administration of glucagon after the development of hypoglycemia leads to no glycemic response. In FDP deficiency, hypoglycemia induced by fasting is accompanied by increases in levels of lactate, pyruvate, and alanine [2, 15, 36], along with acetoacetate and 3-hydroxybutyrate. Hypoglycemia may also be generated by a diet low in carbohydrate and high in protein and fat [10].

Fructose loading yields evidence of fructose intolerance. This test has been employed in patients judged on the basis of the response to fasting to have lactic acidemia, due to a defect in gluconeogenesis (Chapter 49), in order to suggest that the enzyme be assayed on biopsied liver. It should, nevertheless, be undertaken with caution, as there is risk of severe reaction. An intravenous (IV) test is preferred unless hereditary fructose intolerance can be excluded, although patients with FDP deficiency do not develop intestinal symptoms after an oral load [1, 2]. The preferred IV dose is 200 mg/kg [31, 37]. The response is dose-related. A patient became comatose after 500 mg/kg IV [16]. Following fructose, the blood sugar drops to hypoglycemic levels within 15 minutes; lactic acidemia and increased levels of alanine and uric acid accompany systemic acidosis. Fructose administration may induce hyperuricemia and uricosuria even in normal individuals, and the accumulated uric acid results from degradation of adenine nucleotides, following the utilization of ATP in the fructokinase reaction; phosphorus depletion results from the rephosphorylation of adenosine diphosphate (ADP).

These patients are also intolerant of glycerol and sorbitol. Glycerol loading leads to a response similar to that with fructose [1, 7, 30, 38]. Concentrations of phosphate also fall. Sorbitol has been infused to treat cerebral edema; in a child not realized to have FDP deficiency and thought to have cerebral edema, repeated infusions of sorbitol were lethal [28]. Patients with FDP deficiency have normal tolerance of galactose.

TREATMENT

Treatment of the acute episode of hypoglycemia and lactic acidosis is the prompt administration of generous amounts of fluid, sodium bicarbonate, and glucose. The episode usually responds readily. Avoidance of fasting is an important element of subsequent management and if the oral route is temporarily compromised by vomiting, or intercurrent illness, an IV supply of glucose is mandatory.

Table 49.1 Carbohydrate content of common medications (modified from Bosso *et al.* [39])

Medication	Presentation	Sugar form	Manufacturer
ADC	Drops	Sucrose	Parke-Davis
Actifed	Syrup	Sucrose	Burroughs-Wellcome
Amcill 250	Suspension	Sucrose	Parke-Davis
Benadryl	Elixir	Sucrose	Parke-Davis
Betapen-VK	Solution	Glucose	Bristol
Cascara	Liquid (FE 536)	Sucrose	Parke-Davis
Chlor-Trimeton	Syrup	Sucrose	Schering
Compazine	Syrup	Sucrose	Smith, Kline and French
Compocillin-VK drops	Drops	Sucrose	Abbott
Dilantin-30	Suspension	Sucrose	Smith, Kline and French
Erythrocin	Drops	Sucrose	Mead Johnson
Fer-in-sol	Syrup	Sucrose	Abbott
Gantrisin	Syrup	Sucrose	Roche
Ilosone 125	Liquid	Sucrose	Lilly
Keflex	Suspension	Sucrose	Lilly
Lomotil	Liquid	Sucrose	Searle
Phenobarbital	Elixir	Sucrose	Lilly
Polycillin	Suspension	Sucrose	Philips Roxane
Robicillin VK 125 solution	Solution	Sucrose	Robins
Robitussin	Syrup	Glucose and sucrose	Robins
Sudafed	Syrup	Sucrose	Burroughs-Wellcome
Tylenol	Elixir	Sucrose	McNeil

Dietary fructose and sucrose are avoided. In most patients, they do not have to be absolutely eliminated. Rather, the individual tolerance of the patient can be explored cautiously [14], while soft drinks that provide a sucrose or fructose load should be avoided. Lists are available [37] that provide the sugar content and its nature of medicinal liquids. An abbreviated list is shown in Table 49.1. Patients, families, and physicians should particularly be warned about antibiotic elixirs or tylenol syrup which tend to be prescribed when the patient is already metabolically compromised with infection and vomiting-related fasting. A snack at bedtime is often useful, as is uncooked cornstarch (Chapters 39 and 59).

With treatment, hepatomegaly recedes. Subsequent episodes can largely be avoided or aborted. Tolerance to fasting improves with age [1]. Long-term prognosis may be excellent [33]. One patient developed gout at 32 years of age and was treated with allopurinol [33].

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50

Deficiency of the pyruvate dehydrogenase complex

MAJOR PHENOTYPIC EXPRESSION

Acute, potentially lethal metabolic acidosis, hyperventilation, Leigh syndrome, hypotonia, ataxia, failure to thrive or developmental impairment, elevated concentrations of lactic and pyruvic acids and alanine in blood, urine, and cerebrospinal fluid (CSF); and defective activity of the pyruvate dehydrogenase complex.

INTRODUCTION

The pyruvate dehydrogenase complex (PDHC) is a mitochondrial multienzyme system that catalyzes the oxidation of pyruvate to CO_2 and acetylCoA and concomitantly generates reduced nicotinamideadenine-dinucleotide (NADH) (Figure 50.1) [1]. Cofactors include thiaminepyrophosphate (TPP), lipoic acid, coenzyme A (CoA), flavineadeninedinucleotide (FAD), and nicotinamideadeninedinucleotide (NAD1); Mg is required. There are eight different protein components, in seven of which human deficiency disease has been documented. The three basic components E1 (pyruvate dehydrogenase, PDH) E2 (dihydrolipoamide acetyltransferase) and E3 (dihydrolipoamide dehydrogenase) are functional catalytic proteins, of types that are shared by all oxoacid dehydrogenases. There are two regulatory components, E1kinase and phospho-E1-phosphatase with thiamine pyrophosphate (TPP) as a cofactor.

The reaction catalyzed by E1, the first enzyme in the complex (EC 1.2.4.1), which has been referred to as pyruvate decarboxylase (PDC) and contains TPP, accomplishes the oxidative decarboxylation of pyruvate to CO_2 and the linkage of the remaining two-carbon unit to TPP to form a hydroxyethylthiamine pyrophosphate attached to the enzyme (TTP-E1). The E1 enzyme is a heterotetramer of two E1α and the E1β subunits.

The second enzyme, E2 (EC 2.3.1.12), dihydrolipoyl transacetylase, is an acyl transferase; it catalyzes the transfer of the hydroxyethyl group and its oxidation to acetylCoA (Figure 50.1). Concomitantly, the disulfide bridge of the lipoic acid moiety attached to E2 is reduced to the SH form. This attached dihydrolipoic acid is reoxidized in the reaction catalyzed by the E3 enzyme, dihydrolipoyl dehydrogenase or lipoamide dehydrogenase (EC 1.6.4.3). The same E3 component is shared by 2-oxoglutarate dehydrogenase and the branched-chain ketoacid decarboxylase, providing a mechanism for patients who have defective activity in all three systems [2]. In PDHC, a lipoyl-containing catalytic protein has been referred to as protein X, which also functions in acyl transfer [3, 4]. Lipoic acid is attached to the E2 or X protein covalently to lysine moieties. Protein X may also serve in binding E3 to the rest of the complex [5].

Regulation of PDHC involves covalent modification of the protein to produce active and inactive forms. The active form is the dephosphorylated one, and this reaction is catalyzed by a specific phosphatase (PDH phosphatase). A few patients, including an infant with fatal infantile lactic acidemia, have been described in whom the inactive enzyme could not be dephosphorylated because PDH phosphatase activity was deficient [6]. Phosphorylation is catalyzed by a specific kinase, PDH kinase. Additional regulation of PDHC is via end-product inhibition by NADH and acetylCoA [1, 7]. Insulin also activates the enzyme, by fostering the prevalence of the dephosphorylated form. Dichloroacetic acid (DCA) inhibits the kinase, thus keeping the gate to the citric acid cycle locked in the open position [8].

The E1 enzyme is a tetramer of α and β subunits in an $\alpha_2\beta_2$ form. The α protein is 41 kDa and the β is 36 kDa. It is the E1α subunit that is phosphorylated and dephosphorylated by the kinase and phosphatase at serine residues [9].

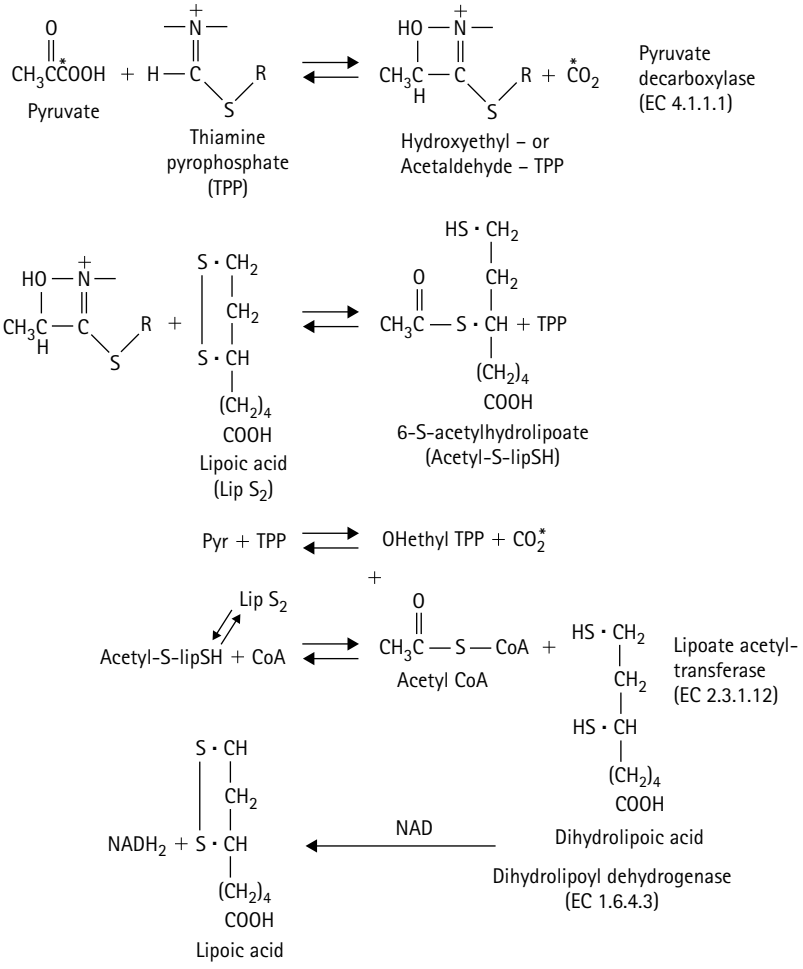

Figure 50.1 The pyruvate dehydrogenase complex (PDHC).

Lactic acid is the major end product of anaerobic glycolysis. It accumulates whenever production of pyruvic acid exceeds utilization. This occurs temporarily during exercise in which there is an oxygen debt, as well as in conditions in which oxygen supplies decrease because of cardiac or respiratory insufficiency or vascular perfusion problems, such as shock. There is also cyclic interconversion of lactate in the Cori cycle in which glucose is converted to lactate in peripheral tissues such as brain and muscle, which oxidize carbohydrates largely to CO_2 and water, and the lactate formed circulates to the liver, where it is converted to glucose [10]. Because of gluconeogenesis, the amount of carbohydrate supplied to peripheral tissues from the liver exceeds the amount ingested. The brain is a major site of its oxidation, and this percentage is greater in infancy and childhood because of the greater proportion of the brain size to body size. The dependency of the brain on oxidative metabolism makes it particularly susceptible to damage in diseases of oxidation that lead to lactic acidosis. The activity of the PDHC is the rate-limiting step in the oxidation of glucose in the brain, and it is central to the normal function of this organ. It has been calculated [11] that the fully activated PDHC could oxidize 180 g of glucose each day; the brain normally oxidizes 125 g a day, leaving little room for error caused by decreased activity of PDHC.

Among genetic deficiencies in PDHC, the most common result from mutations in the E1α gene on the X chromosome. The disease is expressed as an X-linked dominant. Abnormalities in E1β, protein X, E3, and PDH phosphatase are rare. The gene for E1α is at Xp22.1-22.2 [12–14]. The *E1β* gene is on chromosome 3p13-q23 [13]. The *E3* gene is on chromosome 7 at q31-32 [13, 15]. These three genes have been cloned and sequenced [16–18]. The gene for the X protein is on chromosome 11p [19]. A considerable number of mutations has been defined in the *E1α* gene [20]. Most of those in males have been missense mutations, while in females there have been major disruptions in the single affected X chromosome [21, 22].

CLINICAL ABNORMALITIES

Among the defined oxidative abnormalities that lead to lactic acidemia, deficiency of PDHC has been reported to be the most common [23, 24], although this certainly has not been our experience. We have seen many more mitochondrial DNA defects and electron transport chain abnormalities. In a series of 54 patients with deficiency of E1, the spectrum of clinical presentation was as broad as those of the lactic acidemias in general (Chapter 47).

The most severe presentation is of neonatal or infantile metabolic acidosis with lactic acidemia. The acute neonatal presentation may be complicated by hyperammonemia. The majority of these infants die before six months of age, many of them in the neonatal period [25–28].

There is a somewhat more indolent presentation in patients with chronic, more modest lactic acidemia who first come to attention because of delayed psychomotor development [29–36]. Some may present with failure to thrive or poor linear growth. Many of these patients have the clinical and neuropathologic features of Leigh syndrome [37]. As many as 25 percent of patients with Leigh syndrome have been reported to have defects in PDHC [24, 36]. Many of these patients die between ten months and three years of age. They may experience rapid deterioration or episodic deterioration following infections. Patients may have dystonia and, ultimately, they develop spastic quadriparesis. A variety of seizures includes grand mal, myoclonic, absence, or akinetic convulsions. With progression, brainstem abnormalities become prominent. There may be ocular movement abnormalities and central respiratory failure. Death may be from apnea or pneumonia. Another group of patients are characterized by ataxia and lactic acidosis [27].

Neuroimaging may reveal attenuated signal in the basal ganglia, particularly in putamen and globus pallidus [38, 39], and ultimately generalized cerebral atrophy. Histopathologic examination reveals spongiform degeneration and gliosis especially in the basal ganglia. Among the neonatal acidosis group, some have had cortical cysts at autopsy [24, 39]. A number of these patients have also had agenesis of corpus callosum. Cerebral atrophy may be generalized.

The least severe presentations tend to be females with a slowly progressive Leigh syndrome or males with ataxia resembling a slowly progressive spinocerebellar degeneration [40]. Among the group with psychomotor impairment surviving at report, females outnumbered males 2:1 [36]. Among those with the ataxia presentation, some ataxia may be episodic, and some may be induced by carbohydrate and ameliorated by a high lipid diet [41, 42]. A rare presentation is with peripheral neuropathy of infantile origin associated with hypotonia and absent deep tendon reflexes [43]. Episodic weakness has been described in a patient with ataxia and impaired reflexes [11]. Some patients have elevated concentrations of lactic acid in CSF with little or no elevation in the blood [44, 45]: this has been referred to as cerebral lactic acidosis [45].

A group of patients has been described in which dysmorphic features signified prenatal onset of effects of deficiency of PDHC (Figures 50.2–50.4) [24, 46–48]. Our two patients [46], who were siblings, displayed virtually complete absence of psychomotor development. Their phenotype was quite similar to the patient of Farrell and colleagues [48]. Characteristics were wide separation of the eyebrows, epicanthal folds, depressed nasal bridge, a small nose with anteverted flared nostrils (Figures

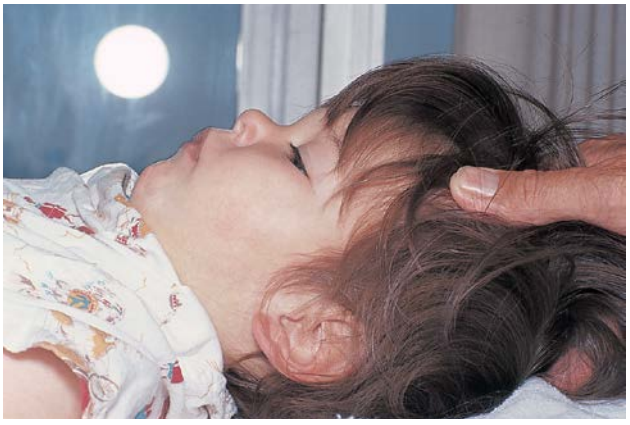

Figure 50.2 LR: An infant with lactic acidemia and deficiency of the PDHC. In addition, she had frontal bossing, a depressed nasal bridge, and an anteverted nasal tip. The ear was large and unusual in shape.

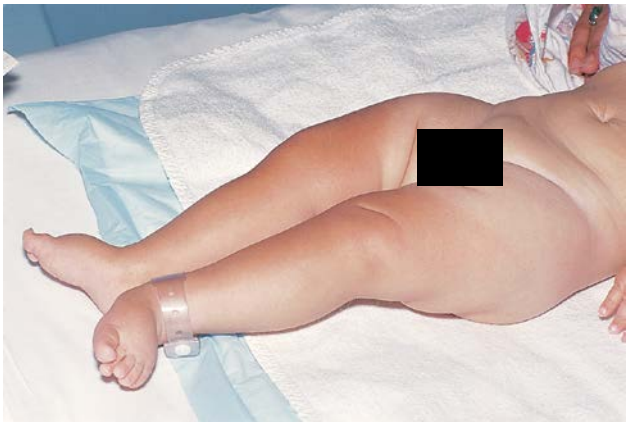

Figure 50.3 LR: The lower extremities were in extreme external rotation.

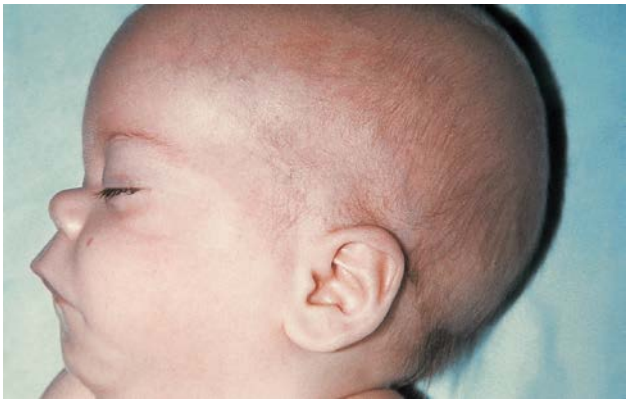

Figure 50.4 Infant with the PDHC deficiency [48], whose face and head had a similar appearance to the infant in Figure 49.2. (Illustration was kindly provided by Dr Richard Wennberg of the University of California, Davis.)

50.2 and 50.4), and a long philtrum. There was limited extension of the elbows and ulnar deviation of the hands. Both hips appeared to be dislocated. The position of the legs was in external rotation so that the feet pointed out like those of a ballerina (Figure 50.3), and abduction was very limited. There was a ventricular septal defect. Visual evoked potentials revealed only a very small degree of cortical response. The facial features have been considered to resemble those of the fetal alcohol syndrome [49]. A common mechanism suggested would be low fetal activity of PDHC, a result in the latter condition of inhibition by acetaldehyde.

Two children with missense mutations in the E1α (PDHA1) presented with dystonia [50]. In a four-year-old boy, treatment with DOPA/carbidopa was effective. He and his sister were diagnosed after findings of elevated lactate in the CSF. PDH activity of cultured fibroblasts was reduced.

GENETICS AND PATHOGENESIS

Deficiency of E1α, the only common form of abnormality in PDHC, behaves as an X-linked dominant character in which, depending on the mutations, there may be quite severe disease in the female. All other defects are autosomal recessive. Reduced conversion of 1-^{14}C-pyruvate to CO_2 has been demonstrated in the fibroblasts of parents consistent with heterozygosity [51], but in four obligate heterozygotes, assay of PDHC revealed somewhat lower than normal levels in two, and normal levels in two. The variability of enzyme assay makes prenatal diagnosis, as well as heterozygote detection, unreliable. In those families in which the mutation has been identified, prenatal diagnosis and heterozygote detection can be pursued with molecular methodology.

Deficiency of PDHC can be documented by assay of a variety of tissues; it is most often accomplished by the study of cultured fibroblasts [23, 24]. The simplest procedure is to measure the conversion of 1-^{14}C-pyruvate to $^{14}CO_2$. Considerable variability has been the rule in the assay, and it has not been possible to correlate the amount of residual activity with the severity of the clinical phenotype. The substrate itself is unstable [52] and activity is also influenced by the methodology employed for disrupting the cell [53]. Assays generally take advantage of the use of dichloroacetate to inhibit PDH kinase and maximize the proportion of the active PDH enzyme [53, 54]. Localization of the defect to the E1 component has generally been done [55] by incorporating ferricyanide into the reaction mixture as an artificial electron acceptor to oxidize the hydroxyethylthiamine and regenerate active E1 enzyme. Deficiency of activity of PDHC has been reported to range from 3 to 40 percent of the control level [23, 24].

There is a tendency for those with severe neonatal disease and dysmorphic features to have virtually no enzymatic activity [48], and for most patients with indolent disease to have considerably more residual enzyme activity [23], but overall the correlation between survival and measured activity has not been good. In general, the severity of disease also correlates with the height of the lactic acidemia; in the fatal neonatal patients, it is very high. Studies of the kinetic characteristics of the enzyme in patients have been few. In one, the PDHC of autopsied liver was difficult to activate [56]. In another [11], the deficient enzyme in muscle biopsied during an acute attack was completely deactivated, although it could be activated *in vitro*.

Antisera have been prepared against purified E1, E2, and E3 [34]. In 19 of 22 patients with deficiency of PDHC in whom the defect was localized to E1, and cellular proteins were labeled with ^{35}S-methionine and immunoprecipitated by antibodies to E1, the deficiency of E1 activity was correlated with a deficient α-subunit [34]. Other patients with deficient E1 activity were immunochemically normal; consistent with simple amino acid substitution resulting in loss of activity. Visualization of PDH proteins by electrophoresis and immunoblotting or immunoprecipitation has revealed decreased or absent E1α protein [36, 57–59], as well as altered migration and an increased phosphorylated form [27, 60]. Whenever E1α is decreased, there is proportional decrease in E1β. Complete absence of E1α and E1β may be associated with fatal neonatal acidosis [27, 36], or with impaired mental development and ataxia [34]. Females with deficient activity and clinical disease have been documented to have two E1α bands [58, 59].

Cloning of the *E1α* gene has permitted extensive documentation of the nature of mutation in this disease. The *E1α* gene has 11 exons spanning 17 kb [16]. It is of interest that a majority of the mutations identified have been in exons 5 to 11; they include the region in which pyruvate is bound to the enzyme (amino acids 130–150), the TPP binding site (170–226), and the serine phosphorylation sites (231–291) [9, 20, 21, 60]. On the other hand, in another series, 12 of 20 mutations were found in exons 10 and 11 [21]. Females with E1 deficiency have two genes, one normal *E1α* gene and one with the mutated gene.

Missense mutation in the pyruvate binding region, a substitution of methionine at position 138 for valine was found in two sisters with 0.6–7 percent enzyme activity and clinical mental impairment who died at 10 and 11 years [20]. Among mutations in the TPP binding area, an alanine-to-threonine 170 change led to a slowly progressive Leigh picture, while a phenylalanine 176-to-leucine change led to death in infancy with severe lactic acidosis [20, 21]. An alanine-to-threonine change at 231, just before the serine phosphorylation site at 232, led to 1.7 percent activity and death from lactic acidosis at 7 days [20]. Similarly, a histidine-to-leucine 263 change just before the serine 264 phosphorylation site gave only 2.5 percent residual activity in a female. An arginine 349-to-histidine mutation very close to the N terminus of the gene occurred in a male with brain atrophy and death at 13 weeks [61]. On the other hand, milder disease was observed in 13 unrelated patients with an arginine 234-to-glycine mutation [20, 62, 63], and 50 percent residual activity was found in two

females with an arginine 273-to-cysteine mutation [62]. A recently discovered tyrosine 243-to-serine change in a patient with neonatal lactic acidemia and bilateral globus pallidus lesions was correlated in fibroblasts *in vitro* with an absence of the normal increase in activity on addition of TPP [64].

The great majority of defects in the PDHC are in the X-linked E1α submit (PDHA1). Expression in females is common. Among clinically affected patients reported, the numbers of males and females may be equal. In males, the majority of mutations has been found in exons 3,7,8, and 11 [65]; R72, R263, and R378 account for half of these patients.

Deletions and insertions have been reported in exons 10 and 11. Many of these patients had severe lactic acidosis and died early in life [61, 63, 66]. An exception was a CAGT deletion at 1167 that produced a protein with 33 extra amino acids and a clinical picture of only exercise intolerance [66]. An unusual mutation in E1α [67], in a family with Leigh encephalomyelopathy presentations, led to alternate splicing in which all of exon 6 containing the TPP binding sites was lost in some transcripts. The mutation, an A-to-G substitution at position 660, did not change the glycine at this position, but led to the loss of exon 6. The mother exhibited normal activity and had 90 percent of normal alleles. A similar mutation in exon 8 of the *HPRT* gene led to classic Lesch-Nyhan disease (Chapter 65). In one of our patients whose mutation led to loss of the last three amino acids, onset was at 16 years, and at 35 years, he displayed ataxia and dysarthria. He was also psychotic. A mutation (R20P) in a patient with Leigh syndrome has been identified in the mitochondrial targeting sequence, altering import of the precursor protein into the mitochondria [68].

In a study of immunoactive enzyme, mRNA, and mutation, three patterns were found: (1) immunodetectable α and β enzymes; (2) no cross-reactive material, but mRNA for both; and (3) absent proteins but deficiency of only α mRNA [62]. It was concluded that failure of expression of one mRNA led to instability of the entire complex. In a boy with microcephaly and developmental delay and a splice site mutation in the *E1α* gene, somatic mosaicism was found with mixtures of normal and variant enzyme [69].

Mutations in the *E1β* gene were found in two unrelated patients [70]. In both, immunoreactive protein was decreased. Both had missense mutations c.395A>G (p.Y132C) and c.1030C>T (p.P344S). Both had severe neonatal onset disease.

Abnormalities in E2 or protein X have been reported in fewer than ten patients [71–73]. A clinical picture of severe neonatal lactic acidosis and hyperammonemia was associated with 24 percent residual activity of PDHC, and 32 percent transacetylase activity [71]. The E2 protein was absent and protein X was reduced. Absent protein X was found in a patient with severe psychomotor impairment, with lactic acidosis and 12 percent activity of PDHC. A boy with an extra band below protein X on immunoblotting with antibody to PDH which was found to be a variant of E2 had an initial presentation of ataxia without impaired mental development, but developed a neuroimaging picture of Leigh disease; fibroblasts displayed 55 percent of control activity of PDHC [71]. Severe lactic acidosis and absence of the corpus callosum occurred in an infant [72] with an absence of the X component. Two patients with a Leigh syndrome presentation [73] had specific absence of the X protein.

Two unrelated consanguineous patients with episodic dystonia and lesions in the globus pallidus were each homozygous for mutations on the *DLAT* gene which codes for the E2 protein [74].

Component X binds to E3 and is also referred to as the E3-binding protein [75]. In two unrelated patients with homozygous splice-site mutations and neonatal lactic acidosis, the mutations were a G>A mutation at the donor splice site of intron 5 leading to exon 5 deletion, and a G>A transition of the splice acceptor site of intron 8. Neonatal lactic acidosis, severe encephalopathy, and death at 35 days was reported in a girl with a homozygous deletion (620 delC) in the *PDX1* gene [76]. In a girl with a large 3913-bp deletion of the *PDX1* gene involving introns 9 and 10 and exon 10, the patient had developmental delay and spastic paraparesis, but her disease was static until 14 years of age when she developed status epilepticus and was treated with valproate, which was followed by severe metabolic and neurologic decompensation [77]. A novel mechanism for the causation of human disease was found in a 25-year-old man with psychomotor delay and spastic diplegia. He developed recurrent dystonia, which disappeared with institution of a ketogenic diet [78]. He had a p.Q248X mutation on the paternal allele. On the maternal side, there was a 46-kb deletion combined with the integration of a full-length LINE-1 element. A model of template jumping was suggested as the mechanism of retropositional insertion of the full-length element.

Defects in E3 result in deficiency of 2-oxoglutarate dehydrogenase and the branched-chain oxoacid decarboxylase, as well as PDHC. In these patients, lactic acidemia and systemic acidosis developed some months after birth. Elevated levels may be found of the branched-chain amino acids and of 2-oxoglutarate, as well as of pyruvate and lactate. In one patient, 2-oxoisocaproic acid was found in elevated amounts. Activity of lipoamide dehydrogenase ranged from 0 to 20 percent of the control level.

Among mutations reported in the *E3* gene, lysine 37 to glutamic acid and proline 453 to leucine were found on the two alleles in a patient with no detectable activity of *E3* [79]. In a boy with microcephaly, impaired mental development, and lactic acidemia, along with recurrent hypoglycemia and ataxia, novel mutations were found in the *E3* gene [80]. They were p.I393T in exon 11 and IVS9+G>A. Pyruvate dehydrogenase activity in fibroblasts was normal.

Abnormalities in the pyruvate dehydrogenase phosphatase gene on chromosome 8q22-23 have been found in a few patients [6, 81]. These include nucleotide

716, leading to p.D239V in a patient who had congenital lactic acidemia and defective activation of PDC by removing phosphate from serine residues in E1α [82]. Among other patients with deficiency of pyruvate dehydrogenase phosphatase, one died at six months of severe metabolic acidosis and lactic acidemia [6], and four had Leigh disease phenotypes [81]. Another patient with deficient activities of this phosphatase, who was homozygous for c.277G>T (p.E93X) [83] had neonatal lactic acidemia and died at 6 months of age. There was no response to dichloroacetate. In two consanguineous brothers [84] with neonatal hypotonia, and lactic acidemia a novel homozygous mutation of 3-bp deleted leucine 213.

TREATMENT

Patients with deficiency of PDHC are sensitive to carbohydrate [41] and may develop life-threatening acidosis when given a diet high in carbohydrates. They respond to the administration of glucose with elevation in concentrations of pyruvate and lactate [41]. The provision of a diet low in carbohydrate and high in fat may lead to reduction in the concentration of lactate and some improvement in the general condition of the patient [26, 41, 85]. In one patient, a diet with 58–66 percent fat was followed by reversal of elevated concentrations of lactic acid and magnetic resonance imaging (MRI) evidence of improvement in the brain [32]. In another patient with E1α mutations, treatment with a ketogenic diet led to reversal of T2 hyperintensity in the globus pallidus [86]. Levels of lactate in the blood may be brought within the normal range using diets in which 50 percent or more of the calories are in fat and 20 percent in carbohydrate. These diets may lead to ketonemia or ketonuria, but not to acidosis or hypoglycemia.

Despite clinical improvement attendant on amelioration of acidotic symptoms, patients with neurologic abnormalities do not usually improve neurologically. These diets bypass the defect by providing the product of the PDHC reaction directly as acetylCoA from the metabolism of fat. In addition, the lesser load of carbohydrate provides smaller amounts of pyruvate to accumulate behind the block and cause lactic acidosis. Caveats raised concerning the use of these diets [87] included the possibility that they could be high in protein and could cause hypercalciuria or kidney stones, as have been observed in patients with convulsions treated with ketogenic diets [88, 89], but the only potentially adverse effect observed in the patients with deficiency of PDHC reviewed was hyperuricemia.

Since TPP is an integral component of the E1 enzyme, high doses of thiamine (100–600 mg/day) have been employed in the hope that a decreased affinity for the cofactor could be overcome by increasing its concentration. Improvement has been reported in a patient described as thiamine-dependent [25]. In other patients, a trial of thiamine is worthwhile in the hope of stimulating residual activity of PDC.

Dichloroacetate effectively reduces levels of lactate in most patients, consistent with the presence of residual activity in PDH in most of them. Intuitively, one might not expect to achieve much in the way of clinical improvement in neurologic features of this disease by treatment with DCA, but we have encountered some dramatic improvements in some patients with PDHC deficiency. Responsiveness to DCA in cultured fibroblasts has been correlated with genotype in severe E1α deficiency [90]. Appreciable increase in PDHC activity in the presence of DCA was found in cell lines with R378C and R88C mutations, consistent with reduced degradation of polypeptides with reduced stability. Carnitine and coenzyme Q are employed in many centers in the treatment of patients with deficiency of PDHC. The usual dose of coenzyme Q is 4 mg/kg, although we are now measuring levels of coenzyme Q, and in deficient patients, often those with electron transport defects, we have employed 10–20 mg/kg, and some use considerably more.

Preincubation of cells from patients with dichloroacetate or calcium [84] restored activity of the complex.

Phenylbutyrate inhibits pyruvate dehydrogenase kinase (PDK) thus inhibiting in-activation of PDHC [91]. Dichloroacetate (DCA) also inhibits PDK. A combination of the two, yielded greater activity than each alone.

In the management of the acute episode of lactic acidosis (Chapter 47), the usual approach is to provide large quantities of intravenous water and electrolytes in the form of $NaHCO_3$. Stacpoole [92] has argued a case against the use of bicarbonate in lactic acidosis, at least in those adult patients with secondary lactic acidemia in intensive care units (ICUs). He cited evidence that infused bicarbonate forms carbon dioxide, which may diffuse across the blood–brain barrier, lowering the pH of CSF; as well as evidence, in experimental lactic acidosis in animals of decreased cardiac output and increased intestinal formation of lactic acid with bicarbonate infusion as opposed to saline infusion. However, these experiments studied the hyperosmolar 1 molar $NaHCO_3$ solution used in ICUs compared with isotonic solutions of NaCl. They may provide an argument for the use of isotonic $NaHCO_3$ in the management of acidosis.

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51

Mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS)

MAJOR PHENOTYPIC EXPRESSION

Mitochondrial myopathy, shortness of stature, stroke-like episodes, seizures, encephalopathy progressive to dementia, migraine, diabetes mellitus, lactic acidemia, ragged red muscle fibers, and the mutations 3243G>A and others in the mitochondrial tRNAleucine gene and other mitochondrial: DNA mutations.

INTRODUCTION

Mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome was first defined as such by Pavlakis and colleagues [1] in 1984, though patients have doubtless been reported earlier. Among the mitochondrial myopathies, this is one of the more common [2].

The typical clinical presentation includes all the features that make up the name of the syndrome, but there is enormous variability. Some affected individuals only have diabetes, or only migraine. Others only have hearing loss, or hearing loss and diabetes [3]. The disease is inherited in a maternal pattern, and the gene is on the mitochondrial genome (Figure 51.1). Most of the patients have had one of three-point mutations in the mitochondrial gene for the leucine (UUR) tRNA A3243G, A3252G and T3271C (Figure 51.2) [4–7]. The A3243G is the most common mutation.

CLINICAL ABNORMALITIES

There is a considerable variety of expression consistent with the varying heteroplasmy of mitochondrial inheritance. The typical picture is of normal development followed by a severe, progressive encephalomyopathy. Onset may be myopathic with exercise intolerance or weakness (Figure 51.3). Many patients have shortness of stature and this may be the first manifestation of disease (Figure 51.4). One of our patients had been treated unsuccessfully with human growth hormone by a pediatric endocrinologist; this has also been reported by others. In many patients, the onset of symptoms is with the first stroke-like episode, usually between four and 15 years, certainly before 40 years and

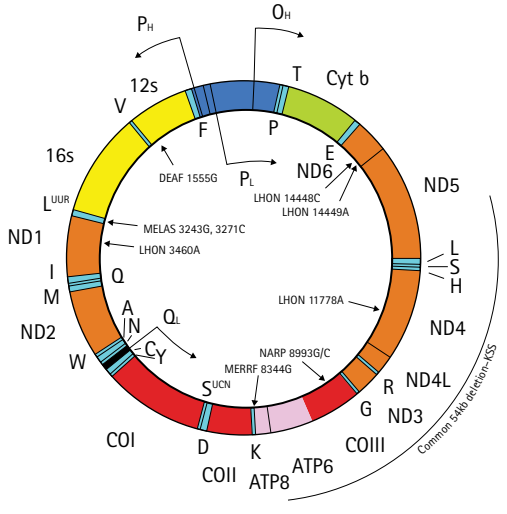

Figure 51.1 The circular DNA of the human mitochondrial genome. Shown are the sites of the mitochondrial genes, as well as the sites for the most common mutations, including the A3243G and T3271C mutations associated with mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome.

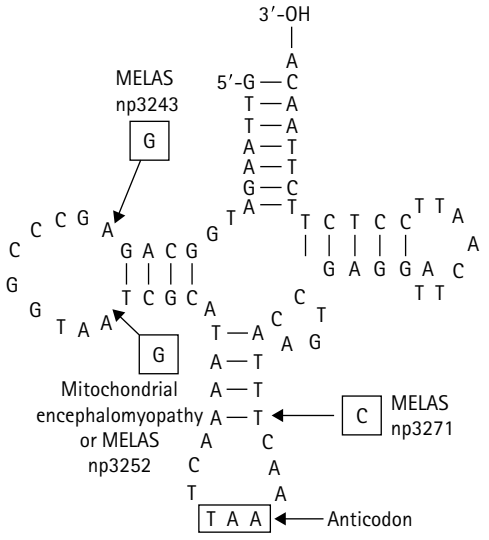

Figure 51.2 The tRNA for leucine, the site of the defect in the mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. In addition to the point mutation at npA3243G, the common mutation in MELAS, and npT3271C and npA3252G the other MELAS mutations, there are a number of other known mutations in the tRNA leucine which cause mitochondrial diseases. These include npT3250C (mitochondrial myopathy), npA3751G (chronic progressive external ophthalmoplegia [CPEO] proximal weakness, sudden death); npA3260G (adult onset hypertrophic cardiomyopathy and myopathy), npA3302G (mitochondrial myopathy), and npC3303T (adult onset hypertrophic cardiomyopathy and myopathy).

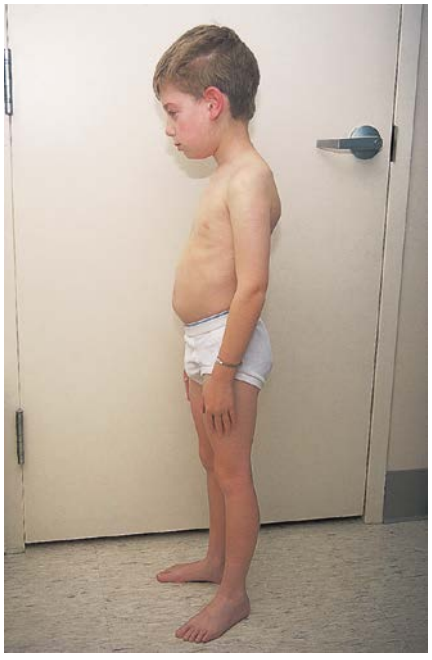

Figure 51.3 KS: A boy with mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) illustrating his lordotic, myopathic posture. He presented at four years of age with weakness and exercise intolerance. He also had insulin-dependent diabetes mellitus. Blood concentration of creatine phosphokinase (CPK) was 462 IU/L. Plasma lactate was 93.1 mg/dL. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

often triggered by infection or physical exercise. [1, 4, 8–14]. Less commonly, the onset of disease may be in infancy [8], often with delayed developmental milestones or learning disability.

Encephalopathy is characterized by seizures and may be accompanied by dementia [14]. The myopathy may be present before the first stroke. At one extreme is a floppy infant at four months of age [8]. More commonly, there is exercise intolerance, easy fatiguability, or frank weakness. Patients may have difficulty going up stairs. Myopathy may be progressive. Proximal muscles tend to be more involved than the distal [8]. Musculature is generally thin. The facial appearance may be myopathic [15]. The creatine phosphokinase (CPK) activity in the blood may be elevated [13, 16]. Some patients have been diagnosed as having polymyositis [11]. The electromyogram (EMG) may demonstrate a myopathic pattern.

The stroke-like episode is the hallmark feature of this syndrome, occurring in over 80 percent of patients [14]. At the same time, these episodes may occur in only a few members of a pedigree, in which a much larger number have the same mutation [15, 16]. In one series of four families [16], stroke-like episodes occurred only in the probands. Two of the affected mothers were clinically entirely normal. In other pedigrees, no member may have had this defining manifestation. The episode may initially be manifest by vomiting and headache, convulsions, or visual abnormalities [8]. Less commonly,

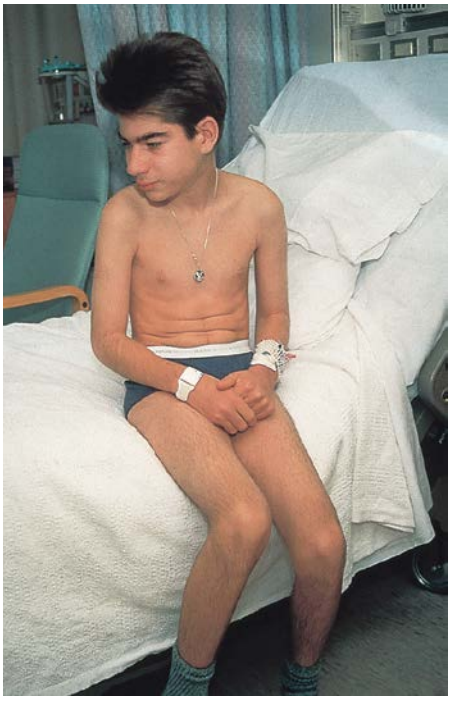

Figure 51.4 NF: A boy with mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) who had strokes on three occasions and had become demented. Stature was very short. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

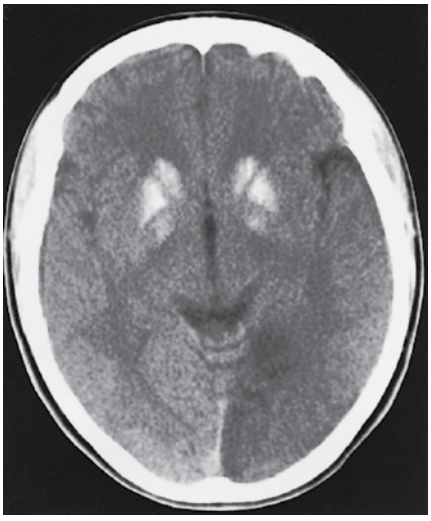

Figure 51.5 CT scan of the brain of MR, a boy with the A3243G mutation, illustrating the posterior infarct and the extensive calcifications in the basal ganglia, including the caudate, putamen and globus pallidus. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

there may be numbness, hemiplegia, or aphasia. There may be recurrent episodes of headache or vomiting lasting from a few hours to several days. The episode may be followed by transient hemiplegia or hemianopia lasting a few hours to several weeks. Computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain following such an episode reveals lucency consistent with infarction (Figures 51.5 and 51.6) [17]. This picture may resolve over hours or days, but later, there may be cerebral atrophy and calcifications, especially in the basal ganglia (Figure 51.6) [17–24]. MR spectrometry reveals decrease in N-acetylaspartate and increased lactate [14] 50 mg.

Infarcts are most common in the posterior temporal, parietal, or occipital lobes, but histologic examination may reveal clear-cut infarcts widely scattered in the cerebrum, cerebellum, or basal ganglia [18, 20, 25, 26]. Thus, these episodes are in fact strokes. The term "stroke-like" may be appropriate in that no vascular changes of inflammation or atherosclerosis are found in the brain and the lesions are not confined to vascular territories. We have tended to refer to this type of lesion as metabolic stroke in other diseases, such as propionic acidemia (Chapter 2) or methylmalonic acidemia (Chapter 3). In MELAS, mitochondrial angiopathy is evident in contrast enhancement in affected areas [21, 27–29] and even in the skin as purpuric lesions.

The migraine or migraine-like headaches seen in these patients may reflect the same process. Headache may be hemicranial. In pedigrees of patients with classic MELAS, there are many members whose only manifestation is migraine (Figure 51.7) [8, 15]. Developmental delay, learning disability [8], or attention deficit disorder [15] is mainly found in patients prior to the development of the first stroke. This was the history of the patient illustrated in Figure 51.4 who did not have his first stroke until the age of eight but had been in a special education program for years. On the other hand, some patients with considerable myopathy and/or other symptomatology may be intellectually normal (Figure 51.3). The encephalopathy, when it develops, may be progressive to dementia (Figure 51.4) in 40–90 percent of patients. The patient may be apathetic and cachectic [18].

Additional neurologic features include ataxia, tremor, dystonia, visual disturbances, and cortical blindness. Some have had myoclonus. Convulsive seizures may be focal or generalized tonic-clonic but may also be myoclonic [7]. The electroencephalogram (EEG) is usually abnormal and there are usually epileptiform spike discharges. Peripheral neuropathy is chronic and progressive.

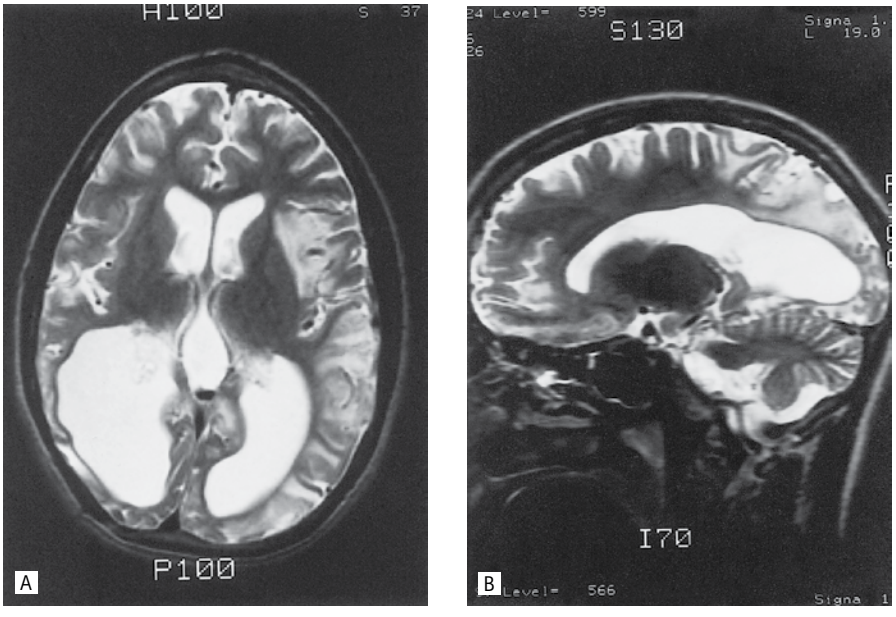

Figure 51.6 MRI of the brain of NF, illustrating widespread cortical atrophy, residual of a right parieto-occipital infarct with ventriculomegaly and increased T_2 signal representing preinfarction state in left temporoparieto-occipital cortex. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

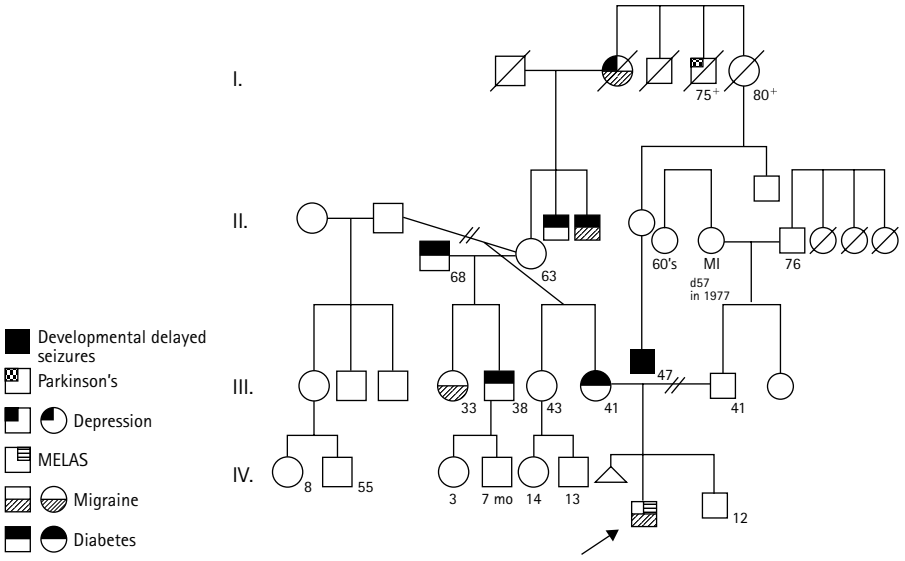

Figure 51.7 Pedigree of the family of NF, illustrating the occurrence of diabetes, migraine, seizures, and other problems. Analysis of the blood revealed the npA3243G mutation.

Some patients have had ophthalmoplegia or ptosis [11]. Others have had pigmentary degeneration of the retina [30] like those with the neurodegeneration, ataxia, and retinitis pigmentosa (NARP) mutation (Chapter 53). Patients have been referred to as having the Kearns–Shy syndrome [11]. Others have presented with the picture of Leigh syndrome (Chapter 53), in which patients have recurrent attacks of neurologic regression, pyramidal and extrapyramidal signs, brainstem abnormalities, and leukodystrophy [31, 32]. Peripheral nerve conduction may be abnormal [33].

An interesting consequence of the MELAS mutation is the occurrence of diabetes mellitus (Figure 51.7) [30]. This appears to be the most common manifestation of MELAS. It is usually type II diabetes [34], but the boy shown in Figure 51.3 had insulin-dependent diabetes mellitus.

Sensorineural hearing loss is another common manifestation and it may be seen in individuals with or without diabetes and no other manifestations of disease [3]. It may also be seen in patients with the classic syndrome. Deafness has been reported in about 25 percent of patients [8]. The disease is a major cause of aminoglycoside-induced hearing loss [35]. This provides an argument for screening for the MELAS mutation in patients with antibiotic-induced deafness, in order to test affected relatives and avoid aminoglycosides in them.

Cardiomyopathy is a less common feature but may be found in about 10 percent of patients. It is usually hypertrophic cardiomyopathy, but it may be dilated [36]. Patients with the MELAS mutations have been found to have MELAS and cardiomyopathy, but others have had isolated cardiomyopathy and no neurologic disease. There may be conduction abnormalities (for instance, Wolff–Parkinson–White syndrome [18]) and often an abnormal electrocardiogram [37]. Huge accumulation of mitochondria has been observed in myocardial fibers [18].

Cyclic vomiting is another presentation [7]. Diarrhea, colitis constipation, intestinal pseudo-obstruction and pancreatitis have also been observed [38]. Pigmentary abnormalities of the skin have been reported [39].

Renal involvement may take the form of renal tubular acidosis, and there may be a typical renal Fanconi syndrome [39]. One patient developed a nephrotic syndrome and had focal glomerulosclerosis [16]. A variety of other organs have been involved in individual patients. One had pancreatitis following valproate administration [15]. Others have had peripheral neuropathy with or without rhabdomyolysis [40]. The histologic signature of the MELAS syndrome is the appearance of ragged red fibers in the muscle (Figure 51.8) [1, 12, 13, 37]. These are best seen in the trichrome stain. In H&E, there may be variation in fiber size and increase in connective tissue. Staining with periodic acid Schiff (PAS), NADH tetrazolium reductase, or for succinic dehydrogenase may show increased subsarcolemmal activity. Electron microscopy reveals an increase in number and size of mitochondria (Figure 51.9), some with paracrystalline inclusion bodies [13, 37].

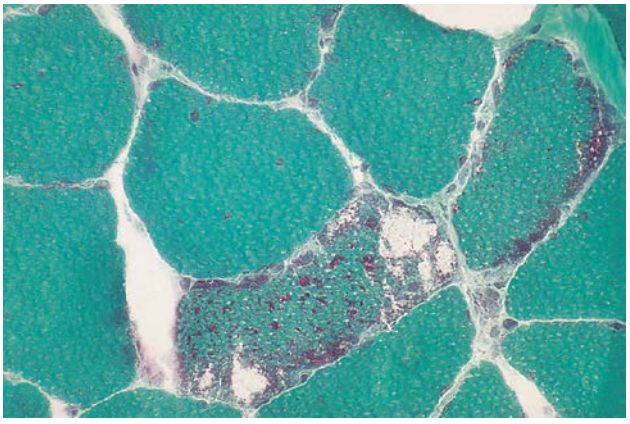

Figure 51.8 Ragged red fibers of the muscle of a patient with mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS). (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

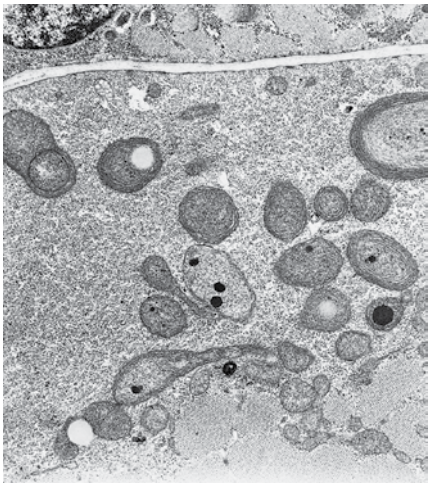

Figure 51.9 Electronmicroscopy of the muscle of the mother of KS. She had diabetes, but no symptoms of myopathy. Illustrated are many pleomorphic mitochondria, abnormal concentric lamellar cristae and electron-dense bodies. There is also glycogen accumulation. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

The lactic acidosis is an important feature of this disorder. It does not usually lead to systemic acidosis and it may even be absent in patients with impressive involvement of the central nervous system. The levels may be elevated in cerebrospinal fluid (CSF) and normal in blood [32]. The patient in Figure 51.4 had repeated determinations of lactate in the blood in the normal range (20 mg/dL); his CSF lactate was 56.3 mg/dL. The CSF concentration of protein may be mildly elevated.

GENETICS AND PATHOGENESIS

The MELAS syndrome is the result of mutation in mitochondrial genes for tRNA [41]. The most common is A-to-G transition at position 3243 of the tRNALeu(UUR) (see Figure 51.1) [4, 5]. Approximately 80 percent of affected individuals have this mutation in the dihydrouridine loop of the gene [8, 16, 42–44]. The other common mutation, occurring in about 8.5 percent of individuals, is also in the tRNALeu(UUR) at 3271 in the anticodon, where there is a T-to-C transversion [7]. The G-to-A transversion at 3252 of the same gene has been reported in mitochondrial encephalopathy [45]. Another mutation in the dihydrouridine loop at nucleotide 3250 is a T-to-C transition [42]. Another mutation in this gene is an A-to-T change at position 3256 [46]. A 5814G in the tRNACys gene was reported in a patient with cardiomyopathy and myopathy [35].

A quite distinct mutation, an A-to-G transition at nucleotide 11084 in the ND4 gene for the subunit of complex I of the respiratory chain, was reported by Lertrit *et al.* [47] in a Caucasian patient. This same mutation was later reported by Sakuta and colleagues [48] in 10–14 percent of Japanese studied, both patients with mitochondrial myopathy and normal controls, suggesting that it might be a polymorphism. On the other hand, this mutation was not found in 109 normal or patient Caucasians nor in American Blacks, nor in a considerable number of patients with other mitochondrial diseases. Thus, the issue on this transition is unresolved. A heteroplasmic mutation in the ND6 gene was found in a seven-year-old whose onset was with vomiting and ketoacidosis, and who went on to develop ataxia, myoclonic seizures, and multiple infarctions [49]. The mutation was c.14453G>A. Mutations were also reported in the *MTTA* and *MTNDS* genes [49]. A c.4332G>A mutation was found in a 47-year-old man with sensorineural deafness who presented with a stroke [50]. A heteroplasmic mutation in the *MTTH* gene was found in a patient with sudden migraine followed by hemiparesis [51]. A large (10.5 kg) deletion was reported in a MELAS patient with a renal Fanconi syndrome [37]. Late onset at 50 years was observed in a patient with the common c.3243A>G mutations whose onset with headaches and seizures was associated with bitemporal lesions [52]. In 23 patients with the common mutation, 77 percent had abnormal peripheral nerve conduction [53]. Autonomic symptoms, such as gastrointestinal symptoms, orthostatic dizziness, and cold or discolored hands and feet were reported [54] in 80 percent of 35 patients with the common mutation [54]. In another approach to relate clinical features with mutation, deafness was the most common feature in 52 adults with the common mutation [55].

The common mutation leads to impaired mitochondrial translation that results in increased synthesis of mitochondrial protein [14]. It creates a new site for the restriction endonuclease *Hae*III leading to a 169-bp fragment in controls after electrophoresis and fragments of 97 and 72 bp in patients with MELAS [43]. Sequencing (Figure 51.10) reveals the G in MELAS where there is an A in control. Varying heteroplasmy among affected individuals appears to reflect variable segregation in the ovum. On the other hand, study of the proportion of mutant DNA in various tissues obtained from a woman and her two daughters revealed similar proportions in tissues derived from ectodermal, endodermal, and mesodermal germ layers, indicating little mitotic segregation after early embryogenesis [56]. The issue of heteroplasmy, which can vary from tissue to tissue making detection difficult has been addressed in MELAS A3243G by the design of peptide nucleic acids which bond to the wild-type mtDNA at 3243 preventing polymerase chain reaction (PCR) amplification and making the mutant the dominant product [57].

Mutations in the tRNA for leucine might be expected to have an important effect on translation and hence protein synthesis in mitochondria. This has been demonstrated in studies of cybrids [25] by fusing human cell lines lacking mitochondrial DNA with exogenous mitochondria containing 0–100 percent of the common 3243 mutant DNA. Cybrids containing more than 95 percent mutant DNA had decreased rates of synthesis and steady-state

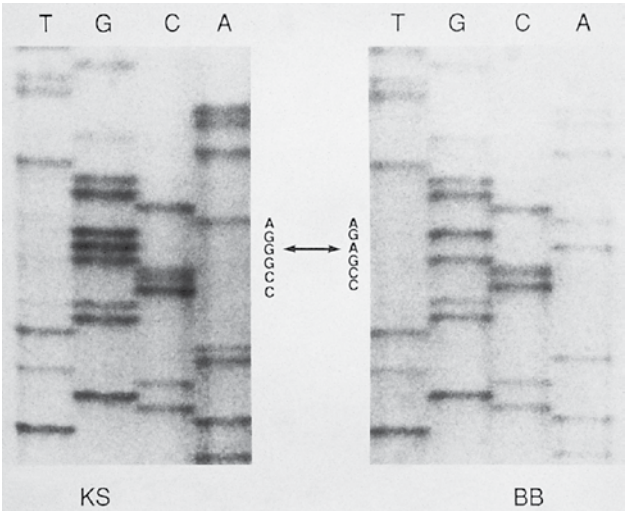

Figure 51.10 Sequencing gel of the mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) region of the leucine tRNA of muscle. The npA3243G mutation was in KS; BB was a normal control. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

levels of mitochondrial proteins leading to respiratory chain deficiency.

Patients with the MELAS syndrome have been found to have marked deficiency in the activity of complex I of the respiratory chain [12]. In mitochondria from muscle, rotenone-sensitive NADH-cytochrome reductase activity was 0–27 percent of control value, and immunochemical study revealed a general decrease in complex I subunits. In a patient with the T-to-C 3250 mutation, complex I activity in muscle was 6 percent of control and that of complex IV was 47 percent of control [58]. The production of CO_2 from labeled pyruvate, malate, and 2-oxoglutarate was in each case reduced [36]. In a study of four patients with the 3243 mutation, the activity of complexes I and IV were reduced in muscle and other tissues, but there was no correlation between the proportion of mutant DNA in a tissue and the activity of the respiratory chain complexes [44].

Mutations in the nuclear gene P0LG (Chapter 56) which codes for mtDNA polymerase γ have been found in patients with a MELAS phenotype [59]. The pathophysiology of stroke in this disease is considered to reflect a mitochondrial role in endothelial or other vascular dysfunction [60].

The A3243G mutations lead to impaired and translation and protein synthesis, resulting in impaired mitochondrial energy production [61].

TREATMENT

A variety of supportive measures is helpful in this disorder, as in other mitochondrial diseases. Riboflavin therapy has been reported to be of benefit in a patient with complex I deficiency and the T-to-C 3250 mutation [58]. A dose of 20 mg twice a day was employed in a two-year-old patient with myopathy who could not ascend stairs and was reluctant to walk. Improvement in muscle strength occurred, and there was no further deterioration over three years of observation.

Coenzyme Q has been reported to be helpful in a number of patients, as has its analog idebenone [14]. Some amelioration of muscle weakness has been observed, as well as some decrease in plasma levels of lactate. CSF lactate did not improve. Doses of 30–90 mg/day were reported [14]. In MELAS, doses as high as 100 mg/kg per day in children or 2.5 g/day for adults have been stated to be required for optimal effects [13, 14].

Experience with dichloroacetic acid (Chapter 53) made clear that levels of lactate can be lowered in both plasma and CSF. MELAS has been one of the disorders that responded temporarily favorably to this agent, but negative effects have also been observed.

Patients with myoclonus may be effectively treated with lamotrigine [59].

Arginine [60–62] has been used intravenously in the acute stroke situation in a dose of 500 mg/kg over 90 minutes in children (10 g/m^2 in adults). A long-term oral maintenance dose was 0.3 g/kg. Plasma concentrations of arginine and citrulline are low in patients with MELAS syndrome. Both are nitric oxide precursors, and it has been proposed that citrulline would be a better source than arginine which requires a transporter to enter the cell. A study of arginine flux and nitric oxide production has been underway in patients treated with either arginine or citrulline [63]. Valproic acid should be avoided, as it may worsen clinical status or neuropathy or induce seizures [64]. Cochlear implants may be useful for deafness.

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Myoclonic epilepsy and ragged red fiber (MERRF) disease

MAJOR PHENOTYPIC EXPRESSION

Myoclonus, ataxia, seizures, optic atrophy, hearing loss, dementia, lipomas of neck and trunk, mitochondrial myopathy with ragged red fibers, lactic acidemia, reduced activity of oxidative phosphorylation, and point mutations in the tRNA lysine and other genes.

INTRODUCTION

Mitochondrial disease and the abnormalities of oxidative phosphorylation were first recognized in 1962 with the description of Luft and colleagues [1] of a hypermetabolic woman with a normal thyroid, who had mitochondria that were abnormal in structure and had loose coupling of oxidation and phosphorylation. The key histologic feature of mitochondrial myopathy, particularly the defects of mitochondrial DNA, was recognized first by Engel and Cunningham [2] with the modified Gomori-trichrome stain that identifies muscle with abnormal deposits of mitochondria as ragged (because of myopathic disruption) red (because of the mitochondria) muscle fibers. The ultrastructural counterpart of this appearance was first recognized by Gonatas and Shy [3], with the description of excessive proliferation of apparently normal mitochondria (pleoconial), greatly enlarged mitochondria (megaconial) or abnormalities in structure with disoriented cristae, or abnormal paracrystalline or osmiophilic inclusions.

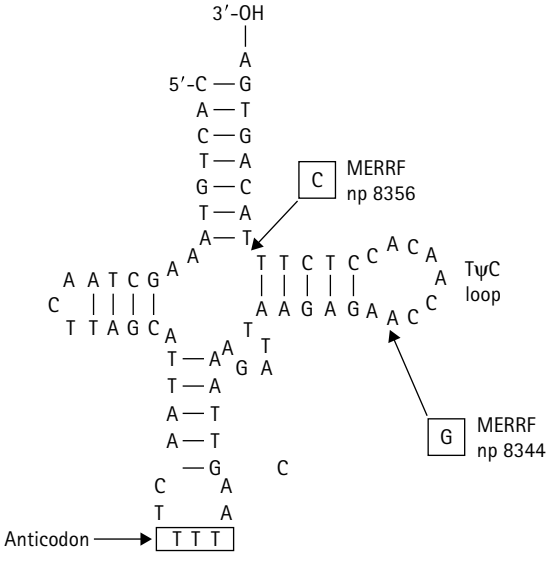

Figure 52.1 The tRNA for lysine and the mutations that cause the MERRF disease.

Myoclonic epilepsy with ragged red fibers (MERRF) was first reported in 1973 by Tsairis and colleagues [4], but current recognition of the disease as a distinct entity was focused by the report of Fukuhara and his associates in 1980 of two patients with myoclonic epilepsy and ragged red fibers [5]. By 1988, 25 examples of the MERRF disease were reviewed by the Columbia group [6]. In the same year, Wallace and colleagues reported evidence that the disease was maternally inherited and qualified as a disease of mitochondrial DNA [7]. This group reported the point mutation in the gene for the lysine tRNA in 1990 (Figure 52.1) [8]. The missense mutation A8344G has been found in approximately 80 percent of patients [8, 9].

CLINICAL ABNORMALITIES

Myoclonic seizures are characteristic features of the disease [10–12]. They have been reported along with ataxia in the absence of ragged red fibers on muscle biopsy in patients with the documented mutation [13]. However, there is considerable phenotypic variability. In a single family with clear maternal inheritance, the clinical picture ranged all the way from an 18-year-old female who had myoclonus, ataxia, deafness, spasticity, and dementia to asymptomatic status with ragged red fiber histology in two members of the family [13]. This is consistent with variable heteroplasmy. The classic picture is of a progressive myoclonic epilepsy, mitochondrial myopathy with ragged red fibers, and slowly progressive dementia (Figures 52.2 and 52.3) [7, 10–23]. Patients may also have akinetic seizures [10] or generalized grand mal seizures [24]. Myoclonic jerks may be virtually continuous and may dominate the clinical picture [10]. The onset of symptoms may be in late childhood or in adulthood [5, 7, 10–12, 14–16, 25, 26]. Truncal and limb ataxia are present, and speech may be scanning. There may be spastic paraparesis, exaggerated deep tendon reflexes in the legs, and extensor plantar responses [10]. Migraine was observed in a 20-year-old woman, who had had panic attacks at 11 years, and developed exercise intolerance, ataxia, hearing loss, and problems of balance [27]. Progressive loss of balance was also observed in a patient who developed seizures and myoclonus at 27 years, and sensorineural deafness at 37 years [28].

Figure 52.2 A 12-year-old boy with the MERRF disease. He could walk, but the muscle weakness is indicated by his stooping posture. His pedigree is that of Family A of which he was the first proband in the paper of Larsson *et al.* [58]; other members of the family had lipomas of the neck. (This illustration and Figure 52.3 were kindly provided by Dr Màr Tulinius of the Department of Pediatrics, Sahlgrenska University Hospital Östra, Göteberg, Sweden.)

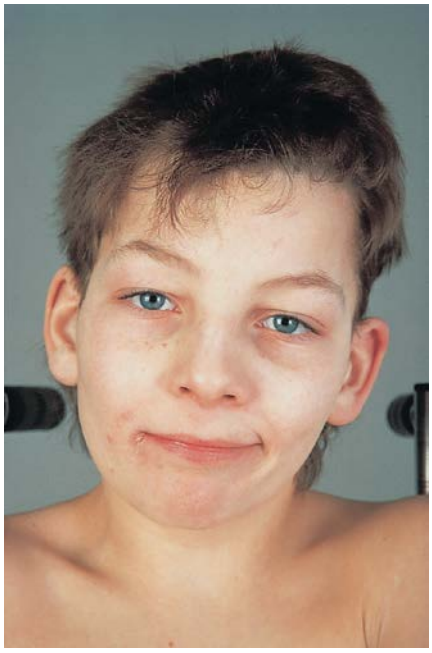

Figure 52.3 The same patient at 16 years of age. By this time, he was wheelchair-bound. He died at 18 years of age. Autopsy information was reported by Oldfors and colleagues [59]. The brain contained mutant mitochondrial DNA in 91–99 percent of tissues studied.

Optic atrophy is common [10]. Eye movements are visually normal, but ocular apraxia has been observed in one patient [25] and abnormal mitochondria have been observed in extraocular muscles [25], along with endomysial fibrosis [29, 30]. Paracrystalline inclusions may be absent. Hearing loss is characteristic [7, 12, 16, 17, 21, 30], but some patients have normal hearing [5, 15, 25, 31]. Stature is usually short [10, 11]. Peripheral neuropathy may be evident clinically; nerve conduction velocity is often reduced [10]. An absence of strokes has been used to distinguish this disorder from mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) (Chapter 51). However, a family has now been reported [32] in which the 20-year-old proband developed sudden migraine followed by left hemiparesis and homonymous hemianopia, as well as seizures. The picture was judged to be that of MELAS. At 25 years, he went on to develop myoclonus and ataxia indicating progression to MERRF. A third-generation Sardinian family also had characteristics of both MELAS and MERRF [33]. An unusual finding seen in a few patients is the occurrence of multiple lipomas in the neck and trunk [24, 34].

The electroencephalogram (EEG) is characteristically abnormal. The typical pattern is of frequent bilateral episodes of high-voltage delta waves early in the disease and bilaterally synchronous bursts of slow spike and wave complexes later [10]. Visual evoked responses (VER) may be reduced [12]. Positron emission tomography of the

brain and ^{31}P-nuclear magnetic resonance spectroscopy of brain and muscle have provided evidence of impairment of energy metabolism and mitochondrial capacity to generate adenosine triphosphate (ATP) [7, 18, 35].

Pathologic examination reveals, in addition to the characteristic myopathy with ragged red fibers (Figure 51.8), widespread neurodegeneration in the dentatorubral and pallidal systems, cerebral cortex, cerebellum, pons, and spinal cord [20, 36–39]. Staining of the muscle for cytochrome oxidase activity may show profound deficiency [10].

Blood concentrations of lactic acid are characteristically elevated, but there are exceptions, as in the case of most features of the disease, and levels are not usually very high [40]. Cerebrospinal fluid (CSF) concentrations of lactate are often higher than those of the blood. The CSF concentration of protein may be elevated, but it is usually normal [11].

GENETICS AND PATHOGENESIS

Inheritance of MERRF disease is maternal; the mutations are predominantly in the mitochondrial gene for the tRNA for lysine. The most common cause is the point mutation at nucleotide 8344 in which there is a G-to-A substitution [8, 16, 35]. This mutation has accounted for 80–90 percent of patients [41–46]. In two families, another mutation, a T-to-C change at 8356, was identified [33, 47]. A different tRNA mutation was reported [48] in a patient with manifestations called a MERRF phenotype and a mutation in the tRNA gene for leucine (MTTL), a C-to-T transition at nucleotide 3256. This patient developed tonic-clonic seizures at 28 years of age. At 45, he had limb myoclonus, mild weakness of neck muscles and deltoids, mild ataxia, and ragged red fibers in muscle. He did not have myoclonic seizures. In addition, he had hypothyroidism following thyroiditis, ptosis, ophthalmoparesis, hearing loss, diabetes mellitus, loss of central vision, optic atrophy, and retinal pigmentary degeneration. A novel mutation in the mitochondrial tRNA phenylalanine (MTTF) was found in a woman with the typical MERRF phenotype [27]. It is clear that mutations elsewhere than in the tRNA lysine can produce the MERRF disease. Mutation in the tRNA histidine (MTTH) has been identified [32], as well as in the two serine tRNAs (MTS1 and MTS2) [49, 50].

A heteroplasmic mutation was identified in the MTTP gene [51] for tRNA proline in a woman with sensorineural hearing loss, seizures, and retinal pigmentary change. A novel mitochondrial DNA mutation (G12147A) was reported [52] in a man with a mixed MELAS/MERRF phenotype.

Abnormalities in tRNA would be expected to lead to impairment of mitochondrial translation and protein synthesis. Consistent with this hypothesis, studies of oxidative phosphorylation in muscle have revealed reduction in the activities of complexes I and IV [7, 46], in which many of the protein components of the complexes are encoded by mitochondrial DNA [53]. Patients with MERRF have also been reported to have defects in complexes II and III [16, 18, 54]. Differences could suggest secondary effects or the substantial problems with methodology in assessing oxidative phosphorylation. On the other hand, phenotypic differences among patients tend to correlate with oxidative phosphorylation capacity of muscle [31].

Mitochondrial genetics differs from nuclear genetics in that the mitochondrial genome is inherited exclusively from the mother. The mitochondrial DNA is transmitted via the cytoplasm of the egg. A cell may contain hundreds of mitochondria; during ovum formation, the number of mitochondria increases while the number of DNAs per mitochondrion decreases to one from two [55]. With growth and development, differences emerge among tissues in mitochondrial content and amounts of mitochondrial DNA. The latter is highest in brain, the organ most vulnerable to diseases of oxidative phosphorylation [56]. Cells may contain more than one sequence of mitochondrial DNA; this is referred to as heteroplasmy.

In MERRF, the rule is for heteroplasmy for the mutation, and there is enormous variation within kindreds in the amounts of mutated DNA and even among tissues in a patient. In oogenesis, there is random segregation of mitochondria with and without mutation into daughter cells, accounting for the difference within a family. Random distribution during cytokinesis leads to different patterns in different tissues. Each tissue appears to have a threshold of production of mitochondrial ATP for adequate cellular function. As the percentage of abnormal mitochondrial DNA increases in different individuals, the threshold is exceeded and clinical disease results. A relatively high proportion of mutant DNA leads to clinical symptomatology [46]. Mitochondrial DNA also has a higher rate of mutation than does nuclear DNA [57]. One of the effects of ageing is an increase in the number of mutations in mitochondrial DNA. Thus, in a family with MERRF, the severity of clinical phenotype correlates with the percentage of abnormal mutant DNA and the age of the individual. Most are phenotypically normal in infancy and childhood. As age-related decrease in oxidative phosphorylation exceeds the threshold for expression in an organ, symptoms of that organ's dysfunction appear, and they become progressively more severe with age [31]. In affected individuals, the greatest percentage of mutant DNA has been found in muscle [53, 58].

For this reason, the work up of a patient thought to be a candidate for this diagnosis may require muscle biopsy. The mutation may be found by analysis of the DNA of lymphocytes or platelets, but the diagnosis cannot be excluded unless the mitochondrial DNA of muscle is analyzed. Rapidly proliferating cells, such as lymphocytes, tend to have lower proportions of mutant DNA, suggesting selection against cells with high mutant content [46].

The MERRF 8344 mutation has been shown to interfere with mitochondrial protein synthesis [53]. When mutant mitochondrial DNA was greater than 85 percent, there was

impressively low synthesis of mitochondrial protein and parallel low levels of complex I and cytochrome oxidase. The effect on translation has been shown by the formation of cybrids, cells into which mitochondria were microinjected. The recipient ρ^0 cells lack mitochondrial DNA and are deficient in dihydrouridine dehydrogenase required for the synthesis of uridinemonophosphate (UMP), and thus they require uridine for growth. Microinjection of human mitochondria permits growth in the absence of uridine, but cells receiving the MERRF 8344 mutation have markedly deficient synthesis of mitochondrial DNA, while those receiving normal mitochondrial DNA synthesize mitochondrial protein well [55].

Family members at risk for maternally inherited MERRF may be tested for the mutation. Most often this is done on blood in those without symptoms. Examination of muscle may be required in those with any symptoms. Prenatal diagnosis is not generally reliable.

TREATMENT

Patients with this disease require supportive therapy aimed at the multiple systems involved. Seizures are managed with conventional anticonvulsant therapy.

Specific therapy is not yet available. Patients with disorders of oxidative phosphorylation, including MERRF, are generally treated with coenzyme Q, because of its place in the electron transport chain (Chapter 47). Doses of 4 mg/kg per day have usually been employed. Others have received riboflavin in doses of 100 mg/day.

Dichloroacetate is effective in lowering concentrations of lactic acid. Clinical improvement in MERRF patients has not been observed.

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53

Neurodegeneration, ataxia, and retinitis pigmentosa (NARP)

MAJOR PHENOTYPIC EXPRESSION

Neurodegeneration, ataxia, pigmentary retinopathy, Leigh syndrome, neurogenic muscle weakness, peripheral neuropathy, and point mutation in the mitochondrial gene for subunit 6 of mitochondrial adenosine triphosphatase (ATPase) (MTATP6), usually a T to G8993, or a T to C8993 transversion.

INTRODUCTION

The 8993 mutation was first described by Holt and colleagues [1] in a family with a maternally inherited neurodegenerative disease in three generations. The major phenotype was of neurogenic muscle weakness, ataxia, and retinitis pigmentosa, and this led to the acronym NARP. The 8993 T-to-G mutation is now referred to as the NARP mutation. In 1992, Tatuch and colleagues [2] reported the occurrence of this mutation in an infant who died at seven months and at autopsy had the typical neuropathology of Leigh syndrome. It is now clear that 8933 mutation is a common cause of Leigh syndrome [3]. A second mutation at position 8993 changing T-to-C in the ATPase 6 gene was identified in a family with Leigh syndrome [4]. Other mutations have been observed. It was noted early that the percentage of mutant mitochondrial DNA varied considerably in heteroplasmic affected individuals, and this leads to considerable variability in phenotypic expression [5]. From the initial description, there was good correlation between the amount of mutant DNA and the severity of clinical manifestations [1].

CLINICAL ABNORMALITIES

The index family was recognized as having a mitochondrial disease not previously described in which there was a variable combination of retinitis pigmentosa, neurogenic proximal muscle weakness, ataxia, sensory neuropathy, developmental delay, seizures, and dementia. There were four patients in three generations. The initial patient was a 47-year-old woman who developed night blindness at 12 years of age and was found to have retinitis pigmentosa; she was nearly blind by 30 years. At 24 years, she had a grand mal seizure and was treated with phenytoin. Unsteadiness in walking was progressive in her thirties. On examination, she had marked ataxia. Ankle jerks were absent and proprioceptive and pain sensations were diminished in the distal lower extremities. Nerve conduction velocity was reduced. Her asymptomatic sister had clumps of retinal pigment and proximal muscle weakness. The daughter of this sister had reduced vision at 25 years and retinitis pigmentosa on examination, along with mild proximal muscle weakness and ataxia, and extensor plantar responses. Her second daughter developed normally until she had a febrile illness at 28 months, in which she was unwell for a month, and she then stopped walking for five months. At three years, she spoke only single words and had pigmentary retinopathy. She was ataxic and had increased tone in the limbs, exaggerated deep tendon reflexes and extensor plantar responses. The electroencephalograph (EEG) was abnormal.

Night blindness is often the first symptom of these patients [6]. This is followed by loss of peripheral vision and, in some, loss of central vision. The index patient at 47 years

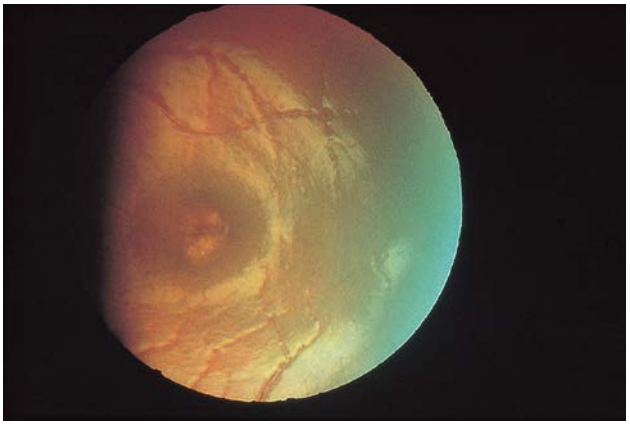

Figure 53.1 AA: A patient with adenosine triphosphatase (ATP) synthase deficiency had pigmentary degeneration of the retina.

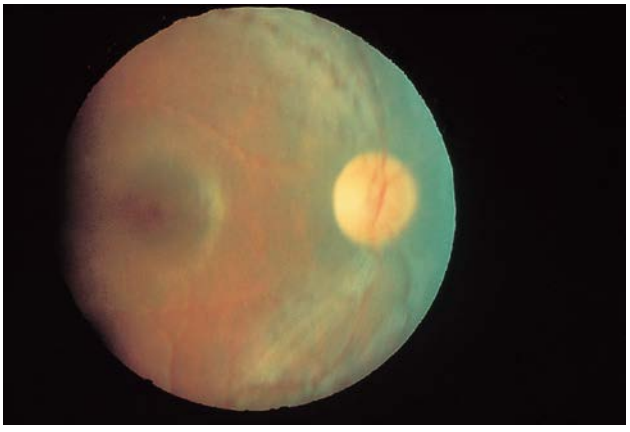

Figure 53.2 AA: The other fundus was also involved. His mutation was in peptide 6.

could just perceive light. Examination of the retina reveals evidence of retinitis pigmentosa (Figures 53.1 and 53.2). The appearance of the clumps of pigment in the retina typically resembles spicules of bone [1, 7]. Some retinas may have a salt-and-pepper appearance [8]. The electroretinogram may be abnormal, as may visual fields. Others have optic atrophy [9]. There may be nystagmus on horizontal or vertical gaze and esotropia [8]. Progression of retinal disease has been described from the appearance of salt and pepper in the retina in the absence of symptoms to constriction of visual fields along with the appearance of bone spicules in the retina, optic nerve pallor, and arteriolar attenuation [10].

Ataxia may be a prominent feature of the disease (Figures 53.3 and 53.4). It may lead to injuries and localized areas of traumatic hyperplasia (Figure 53.5). Cerebellar atrophy has been observed on neuroimaging [9]. Other patients have had dystonia.

Some patients have been impressively hypotonic [9]. Others have had localized proximal muscle weakness but, as recognized in the initial series, the weakness is neurogenic. Muscle biopsy does not show ragged red fibers or abnormal mitochondria [1–3, 9–12]. There may

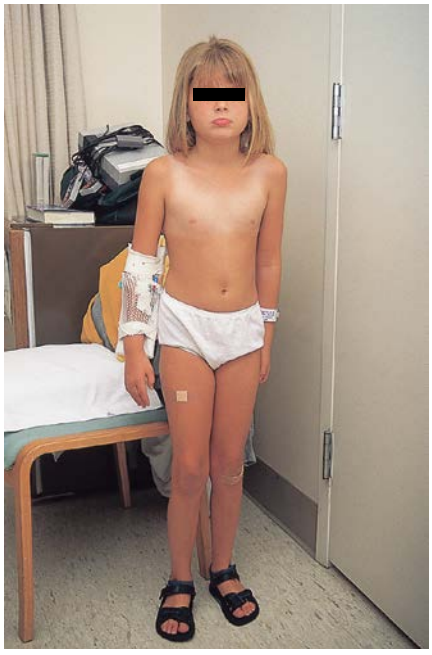

Figure 53.3 BF: A girl with the neurodegeneration, ataxia, and retinitis pigmentosa (NARP) mutation, a deletion at 8993, in mitochondrial DNA. She had been well until approximately one year before when she and her twin brother developed an acute life-threatening episode of which he died.

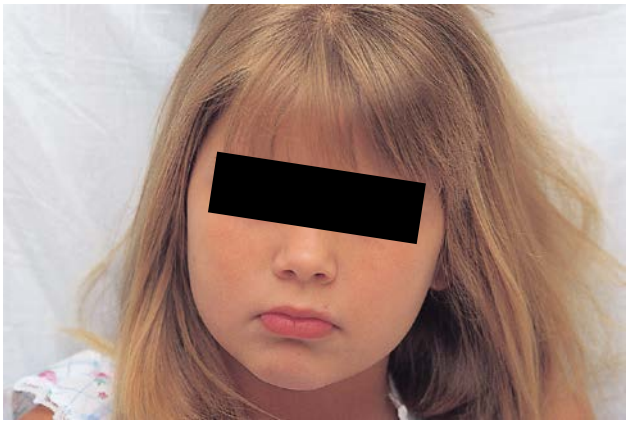

Figure 53.4 BF: She was quite ataxic and fell so frequently she had a raised bony area in her mid-forehead.

be evidence of denervation, lipid droplets, or variation in fiber size diameter. The electromyogram (EMG) is normal.

Peripheral neuropathy may be evident on clinical examination [1]. Nerve conduction velocity is reduced in a pattern of axonal sensory neuropathy.

Seizures may be generalized and associated with spike and wave bursts on EEG [1, 9]. Two patients have had infantile spasms and an EEG pattern of hypsarrhythmia [9]. Others have had myoclonic seizures. Less severely affected patients have had migraine, some with no other manifestation of illness. Others have had depression or bulimia. Some have been developmentally delayed, some of them severely. Hearing loss has been reported [13].

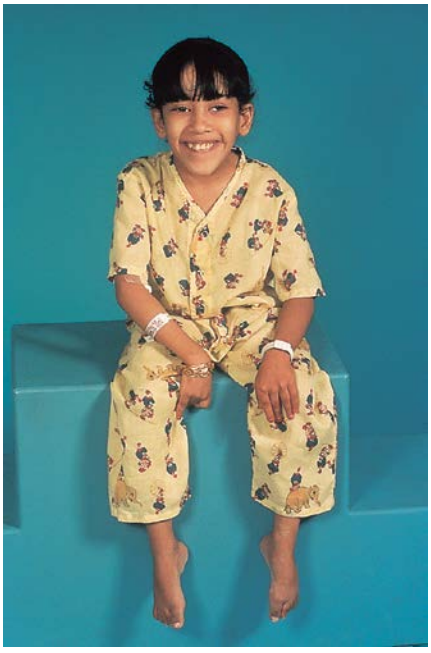

Figure 53.5 TS: A patient with the neurodegeneration, ataxia, and retinitis pigmentosa (NARP) syndrome. She had ataxia and retinitis pigmentosa.

There have been a number of deaths in infancy (see Figure 53.3) [2, 9]. The acute life-threatening episode may be associated with lactic acidemia [2]. The advent of dichloroacetate control of lactic acidemia has permitted us to observe episodes of acute acidemias in patients with the NARP mutation in the absence of lactic acidemia. These episodes of acute acidosis requiring admission to hospital, and parenteral fluid and electrolyte therapy have been characterized by ketoacidosis. An infant who died had lactate levels of 3–5 mmol/L [2]. Others had levels up to 5.2 mmol/L [9]. In one 50-year-old man, with mildly impaired mental development the cerebrospinal fluid (CSF) lactate was normal [9]. CSF concentrations of protein were normal [9]. Some patients have had recurrent vomiting.

An expanded spectrum of NARP syndrome [14] was recognized when the 8993G mutation was found in a patient whose diagnosis on magnetic resonance imaging (MRI) was acute demyelinating encephalomyelitis (ADEM). The patient had neurogenic muscle weakness, and ataxia, typical for NARP, but other affected family members had fewer clinical manifestations. Nevertheless, his mother died of fulminant hepatic failure following valproate administration. In four families studied, none had retinitis pigmentosa. One was referred for "cerebral palsy", attention deficit disorder, and learning disability. Later, he had episodes of ataxia, headache, and peripheral neuropathy following febrile illnesses.

Leigh syndrome (Chapter 47) was first associated with the classic NARP mutation in the autopsy of an infant who died at seven months of age with lactic acidemia, seizures, and apnea [9]. She had been hypotonic and had head lag from early infancy. Neuropathologic examination revealed bilateral cystic lesions of the basal ganglia, thalamus, substantia nigra, and tegmental brainstem. There was proliferation of astrocytes and blood vessels in these areas. A maternal aunt and uncle had died of Leigh syndrome. Another maternal uncle was normal until 12 years of age, when he developed a bout of weakness and ataxia from which he recovered. There were further episodes and he developed retinitis pigmentosa. At 33 years, he was ataxic, legally blind, mentally handicapped, and in an institution.

Of seven patients with typical Leigh spongiform changes on neuropathology or the characteristic appearance on MRI, the classic NARP mutation was found in all [15]. In each, there was heteroplasmy, but the mutation was in high proportion in blood and muscle. It was found in four asymptomatic mothers and two asymptomatic siblings. This series was expanded to 12 patients in ten families, all with the same mutation [3]. Consistent with the observations of Tatuch *et al.* [2], the Leigh phenotype was associated with a high percentage of abnormal mitochondrial DNA. The heterogeneity of Leigh syndrome is pointed up by the fact that the 12 patients of Santorelli *et al.* [3] were found in the study of 50 patients with typical Leigh syndrome. These authors compared 18 patients reported with the NARP mutation and Leigh syndrome with 34 and 64 in whom the underlying disease was cytochrome oxidase deficiency or pyruvate dehydrogenase complex deficiency (Chapter 50), respectively. Smaller numbers had biotinidase or complex I deficiency. Among the features of the clinical picture, only retinitis pigmentosa and positive family history seemed to distinguish the patients with NARP. An earlier-onset, more rapid course or propensity for seizures was more common among the NARP patients. Patients with the classic NARP mutation and Leigh syndrome were also reported by Ciafaloni and colleagues [11], Shoffner and colleagues [8], and Mäkelä-Bengs and colleagues [9]. Thus, it is clear that the 8993 mutation in mitochondrial DNA is a common cause of Leigh syndrome.

The syndrome in these patients is characterized by developmental delay, some after a period of normal development, and hypotonia followed by psychomotor regression; some have had ataxia or dystonic posturing [8]. Spastic quadriparesis has been reported [3]. Brainstem dysfunction leads to ophthalmoplegia, apnea, ventilator dependence, or death [3]. Neuroimaging reveals symmetric areas of decreased density on computed tomography (CT) or MRI in the basal ganglia, brainstem, periventricular and periaqueductal areas (Figure 53.6). Blood concentrations of lactate were increased as high as 7 mmol/L with a mean concentration of 4.6 mmol/L [3]. A CSF concentration of 7.12 mmol/L was reported [11]. Neuropathologic examination revealed reduction in the size of the caudate, globus pallidus, putamen, and cerebellum. Microscopic examination showed gliosis and demyelination of white matter and spongiform changes with relative preservation of neurons in the basal ganglia, thalamus, hypothalamus, and medulla [8]. Some patients have had hypertrophic cardiomyopathy [3].

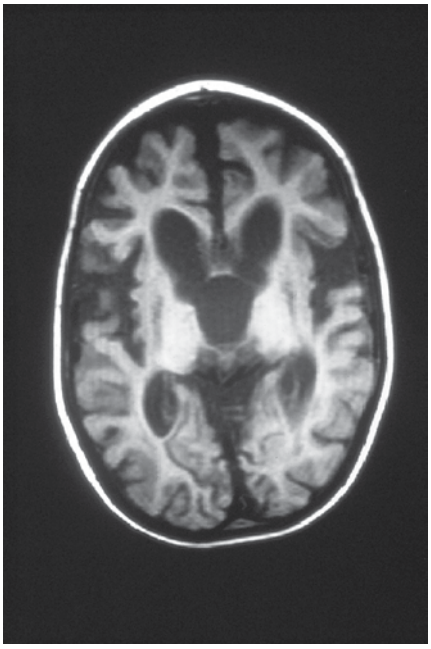

Figure 53.6 AA: CT scan of the brain. There was considerable atrophy. Progression of disease was documented [16] in a woman who demonstrated only retinitis pigmentation and mild sensory neuropathy by the age of 39 and was otherwise clinically normal. At the age of 59 she was blind, diabetic, and had sensorineural hearing loss.

GENETICS AND PATHOGENESIS

The NARP mutation is clearly inherited in a maternal pattern [11, 12, 14, 15–17]. Recurrence of disease in two siblings of a mother with two different mates has been reported [11]. All of the families studied have revealed degrees of heteroplasmy. In general, the correlation between the severity of phenotype and the proliferation of mutant mitochondrial DNAs has been very good [1–3, 9, 11]. Patients with infantile encephalopathy and Leigh syndrome have tended to have over 95 percent of mutant mitochondrial DNA. Later-onset patients have had 80–90 percent, while asymptomatic patients may have had as little as 3–6 percent.

In general, good correlation between degree of mutation and clinical severity has been observed in lymphocytes and fibroblasts [18]. Patients with mutation loads above 95 percent usually present with Leigh syndrome [19]; those with 70–90 percent have the NARP picture. Both phenotypes may be seen in the same family. Correlation has been better with the proportion of mutant DNA in the blood than in the fibroblast. For instance, an asymptomatic mother had 39 percent in blood and 71 percent in fibroblasts [2].

Prenatal diagnosis has been made in two affected pregnancies at risk by examination of chorionic villus samples [20], but, because of the random distribution of mutant DNA, neither prenatal diagnosis nor carrier detection is reliable. Calculations from 56 pedigrees relating severity of symptoms and mutant load should be useful in genetic counseling [5].

There has been some evidence for nonrandom segregation of mutant NARP mitochondria in oocytes, such as a mother with 10 percent mutant DNAs having three offspring with 90 percent or more mutant DNAs. Examination of oocytes has revealed a predominance of mutant DNA. A bottleneck for mitochondrial DNA in embryogenesis might lead to a reduction or enhancement of amounts of mutant mitochondrial DNA [9].

The common mutation was found originally in nucleotide 8993 of the mitochondrial genome when digestion of leukocyte mitochondrial DNA with the restriction endonuclease *Ava*I revealed an unusual pattern of fragments. In involved members, a variable portion of the normal 14.4-kb fragment was cleaved into two, one 10.4 kb and one 4.0 kb. Further digestion with *Pvu*II cleaved the 10-kb fragment into two of 6 and 4 kb, respectively, which localized the gain of the *Ava*I site to the ATPase 6 reading frame. Both normal and mutant populations were found in muscle, as well as blood. Polymerase chain reaction (PCR) amplification with primers 8648–8665 and 9180–9199 revealed a single fragment, which was cleaved by *Ava*I in patients, but not in controls. Sequencing identified the T-to-G change at 8993. This change leads to a change from the highly conserved hydrophobic leucine to the hydrophylic arginine at position 156 of subunit 6 of the mitochondrial H+-ATPase. This would be expected to interfere with the hydrogen ion channel formed by subunits 6 and 9 and lead to failure of adenosine triphosphatase (ATP) synthesis [2]. It did not affect hydrolysis of ATP. A *de novo* insertion in the *MT-ATP* gene led to a truncated subunit and a decrease in the amount of ATP synthase [13]. The patient, a 40-year-old with blindness and optic atrophy was heteroplasmic, 85 percent in muscle and 26 percent in blood.

The activity of the enzymes of the respiratory chain tends to be normal in the frozen muscle of patients [3]. However, Shoffner and colleagues [8] found deficiencies in the activities in muscle of complex I and III. Testing of oxidative phosphorylation revealed a reduction in the rate of generation of ATP [9]. The T-to-C mutation at 8993 replaces the leucine with proline. This would change the helical structure of the protein and would be expected to interfere with proton conduction [21]. Nevertheless, ATP production was not impaired [22]. In general, the 8993C disease tends to be less severe than the 8993G [5].

A patient with classic NARP manifestations had a *de novo* insertion in the MT-ATP6 gene (m.8618-8619insT) which led to a frameshift affecting 31 amino acids before leading to a stop codon [15]. Thus, molecular analysis of a patient with NARP should not be limited to search for 8993T>G/C.

TREATMENT

Supportive treatment includes the management of seizure disorder and the prompt treatment of infection. Experience

with dichloroacetic acid indicates success in reducing levels of lactic acid. Effects on the neurologic features of the disease were unimpressive.

Cultured fibroblasts with the 8993G genotype were protected from death in the presence of gramicidin or oligomycin in a glucose-free medium by incubation with 2-oxoglutarate/aspartate, which increased ATP content [23]. Patients have not yet been treated.

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54

Kearns–Sayre syndrome

MAJOR PHENOTYPIC EXPRESSION

Onset prior to 20 years of age of progressive external ophthalmoplegia, ptosis, pigmentary retinopathy, block in cardiac conduction, ataxia, elevated protein in cerebrospinal fluid (CSF), decreased CSF/serum folate, ragged red muscle fibers, and deletion in mitochondrial DNA.

INTRODUCTION

In 1995, Kearns and Sayre [1] reported a syndrome of retinitis pigmentosa, external ophthalmoplegia, and complete heart block. It has for some time been recognized as an encephalomyopathy with variable neurologic manifestations, including cerebellar ataxia, muscle weakness, sensorineural deafness, and mental deterioration [2]. There may be elevation of the protein in CSF to values over 100 mg/dL. Muscle biopsy reveals ragged red fibers [3]. Lestienne and Ponsot [4], Holt and colleagues [5], and Zeviani *et al.* [6], in 1988, reported deletions in the DNA of mitochondria in biopsied muscle. The common deletion approximates 5 kb. The disease is virtually always the result of spontaneous new mutation. Cerebral deficiency of folate has been reported in this disease, and a favorable response to treatment with folic acid was observed [7].

CLINICAL ABNORMALITIES

Patients with Kearns–Sayre syndrome usually appear normal in early childhood, developing features of the disease in later childhood or adolescence. It is probably an artificial distinction that patients developing signs of disease after the age of 20 years are referred to as having chronic progressive external ophthalmoplegia (PEO), because they may have the same deletions as those presenting earlier and may develop any of the multisystem features of classic Kearns–Sayre syndrome [2].

The earliest manifestation is often a limitation of external ocular movement or ptosis (Figures 54.1 and 54.2). These manifestations are chronically progressive, and the classic appearance is that of bilateral ptosis and ophthalmoplegia. There may be progression to complete ophthalmoplegia. Electromyography (EMG) of the orbicularis oculi muscle may show myogenic changes.

Pigmentary degeneration of the retina may take the pattern of salt and pepper retinopathy in which there are regions of hyper- and hypopigmentation or a bone spicule appearance of retinitis pigmentosa [8]. The Kearns–Sayre triad is ptosis, ophthalmoplegia, and pigmentary retinopathy. There may be optic atrophy. Some patients have had an eventual loss of the pigment epithelium. Others have had a choroideremia pattern in which there is complete choroid atrophy [9]. Supratentorial and cerebellar atrophy have been observed [10].

Visual impairment may be the presenting complaint. Other patients with pigmentary changes on fundoscopy may have normal visual acuity. Electroretinopathy may reveal delayed A waves signifying tapetoretinal degeneration even in patients without symptoms or abnormality visible in the fundus.

Skeletal myopathy may be evident in muscle weakness or exercise intolerance. Deep tendon reflexes may be diminished. Some patients have developed scoliosis. Cerebellar abnormality may be evident in ataxia, a broad-based gait, or dysmetria. There may be an intention tremor. Sensorineural deafness is another common neurologic manifestation of the disease. Dementia may ultimately

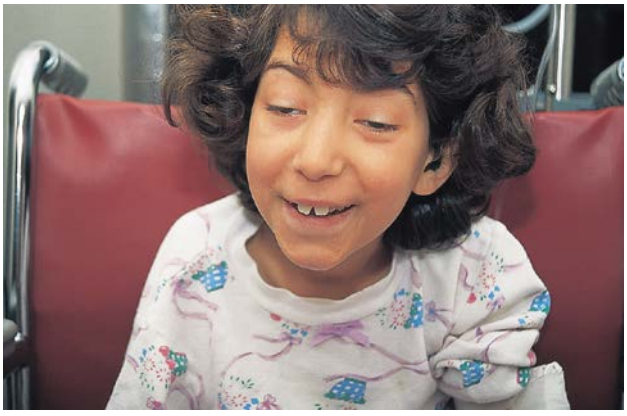

Figure 54.1 GF: A ten-year-old girl with Kearns-Sayre syndrome. She was very short and had pronounced ptosis bilaterally.

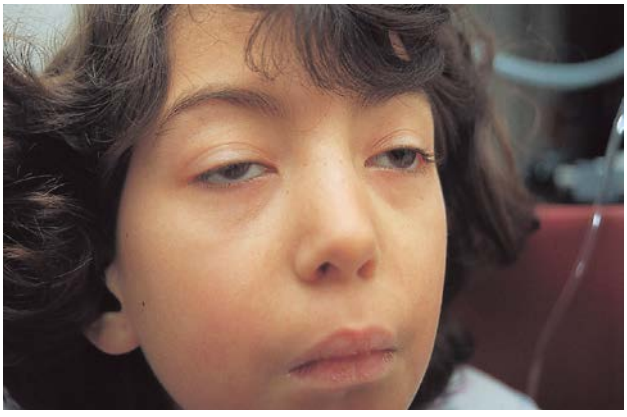

Figure 54.2 GF: When asked to follow a light upwards, the patient demonstrated a paresis of upward gaze.

occur. Muscle biopsy classically reveals ragged red fibers [11] when the specimen is stained with Gomori trichrome. Structural abnormality may be identified by electron microscopy. There may be aggregates of mitochondria [12]. There may be cytochrome c oxidase (COX) negative fibers, deficient pyruvate and malate oxidation and deficiency of complex I and III [7].

CSF concentration of protein is usually referred to as >100 mg/dL, but many patients, even among those with elevated concentrations of protein, have lower levels [11]. Computed tomography (CT) scan or magnetic resonance imaging (MRI) may reveal atrophy of the cerebellum or brainstem [13], and there may be calcifications in the basal ganglia [13, 14]. Some patients have had lesions in the thalamus and brainstem, as seen in Leigh syndrome. Others have had diffuse white matter hypodensities [6]. The histopathology of the brain is that of spongiform degeneration [11].

Cardiac conduction (Figure 54.3) is classically abnormal and typically takes the form of a complete atrioventricular block or a right bundle branch block. The PQ interval may be prolonged on electrocardiogram (ECG), or there may be a prolonged QT [14]. Evidence of cardiomyopathy

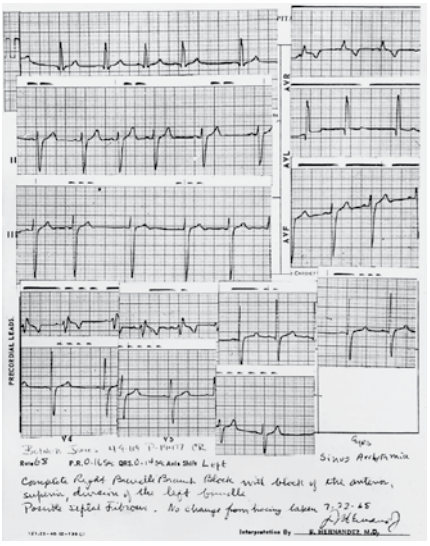

Figure 54.3 Electrocardiogram of a patient with Kearns-Sayre syndrome, illustrating a complete heart block.

has been obtained by biopsy [12]. Clinical evidence of cardiomyopathy can range from tachycardia to frank failure. One of our pediatric patients presented with a seizure resulting from complete heart block [15]. She also had retinopathy and ophthalmoplegia.

Other nonneurologic manifestations include shortness of stature. A variety of endocrine abnormalities have been encountered [2, 16], the most common of which are diabetes mellitus and hypogonadism, including amenorrhea and delayed puberty. Hypoparathyroidism, thyroid abnormalities, and hyperaldosteronism are less frequent [17, 18]. Nonautoimmune Addison disease has been reported [18] in a patient with a 4.9-kb deletion. Hypomagnesemia has been observed [14]. Hypoparathyroidism has been a component of a multiple endocrine abnormality syndrome [19].

An 18-year-old girl was reported [20] in whom hypoparathyroidism and renal tubular dysfunction were associated with inappropriate hyperexcretion of magnesium and potassium. Another hypoparathyroid patient presented at five years of age with recurrent episodes of carpopedal spasms resulting from persistent hypomagnesemia and hypocalcemia with increased urinary fractional excretion of magnesium and calcium [21].

Another syndrome has been reported in four unrelated children with Kearns–Sayre syndrome who presented first with hypoparathyroidism and deafness [14]. Hypocalcemic tetany, a consequence of deficiency of parathyroid hormone, was well controlled by treatment with low doses of 1,25-dihydroxycholecalciferol. Two of three patients had hypomagnesemia.

Renal tubular acidosis is another interesting manifestation [21, 22] which is also seen in other disorders of mitochondrial electron transport function. There may be glycosuria and a generalized amino aciduria [21]. Some presentations have resembled Bartter syndrome or Lowe syndrome [23].

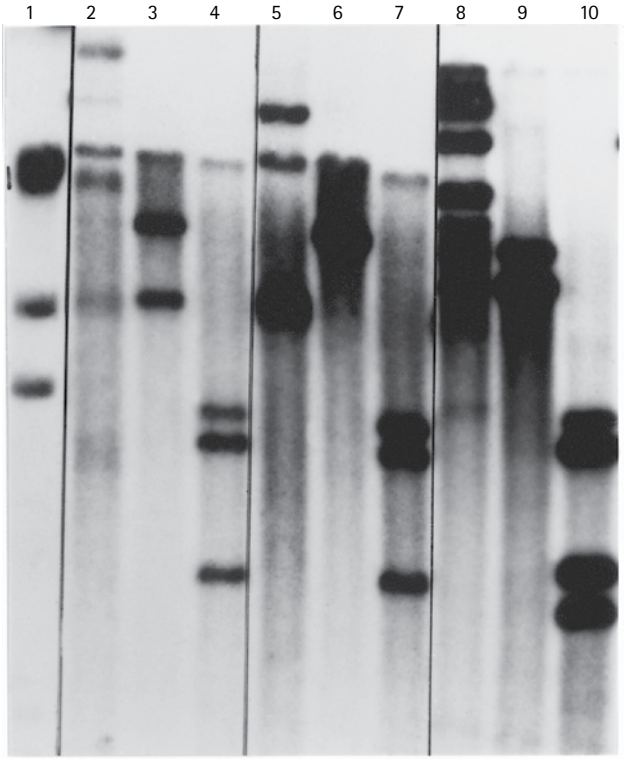

Figure 54.4 Electrophoretic patterns of restriction fragments of mitochondrial DNA from two patients with Kearns–Sayre syndrome (lanes 2–4 and 8–10 and a control individual, lanes 5–7. Lanes 2, 5, and 8 were uncut, lanes 3, 6, and 9 cut with *Bam*HI, and lanes 4, 7, and 9 were cut with both *Bam*HI and *Eco*RV. Lane 1 represented size standards. The patient shown in lanes 8–10 had the most commonly encountered deletion. (Reprinted from *Molecular Genetics and Metabolism* [15] with permission from Elsevier.)

Lactic acidemia is not a predominant feature in many patients, but some have had lactic acidosis; some have mild elevations of levels of lactic acid in the blood. In others, the CSF concentration of lactic acid is elevated in the absence of lactic acidemia. CSF protein may be elevated [7]. The ratio of CSF/serum folate was reduced (0.6, control 1.5-3), along with [7] profound deficiency of 5-MTHF. Low levels of CoQ10 have been reported [25] in serum and CSF fluid.

An unusual presentation is with the Pearson-marrow syndrome (Chapter 55). Patients with the Pearson syndrome have the same area of deletion as Kearns–Sayre syndrome, and patients who have survived the early morbidity of the Pearson syndrome and whose marrow dysfunction resolved have been reported to go on to develop Kearns–Sayre syndrome [24].

GENETICS AND PATHOGENESIS

Kearns–Sayre syndrome is virtually always sporadic [11], suggesting that the deletion in the mitochondrial genome occurred in the formation of the affected individual. Many mothers of affected individuals have been studied without finding deletions. Analysis of mitochondrial DNA has revealed multiple deletions in muscle. These deletions could not be found in rapidly dividing tissues, such as leukocytes or cultured fibroblasts. A similar pattern of multiple deletions in mitochondrial DNA was seen in a study in which there were two affected brothers and first-cousin parents, a pattern that suggested autosomal recessive inheritance [26, 27].

The vast majority of patients with Kearns–Sayre syndrome have deletions spanning approximately 5 kb and referred to as the common deletion (Figures 54.4 and 54.5). Many different deletions have been observed in the O_H to O_L arc (see Figure 55.1). In about half of patients, this is a 4.9-kb deletion extending from the NAD dehydrogenase (ND5) to the ATPase subunit gene [28]. This is an area in which there are 13-bp direct repeats on either side, np 13,447 to 13,459 and np 8470 to 8482 [29–31]. It appears likely that this produces a situation in which hot spots promote deletion. Deletions in this area remove structural genes and some tRNA genes, which would interfere with mitochondrial protein synthesis. Overall deletions have ranged from 1.3 to 7.6 kb [17, 18]. The proportion of mutated genomes ranged in these series from 27 to 85 percent of total mitochondrial DNA. In some patients, the proportions in different tissues were very variable. In general, the proportion of abnormal DNA increases with age, paralleling the worsening of clinical manifestations. The deletions are all large enough to be readily distinguished from control by digestion with restriction endonucleases and electrophoresis on agarose gel (Figure 54.5). Southern blots display a 16.5-kb band in normal individuals and smaller bands in those with deletions.

A 4.9-kb deletion and heteroplasmy has been observed in wild mice [32]. It has been thought that deletions in a region

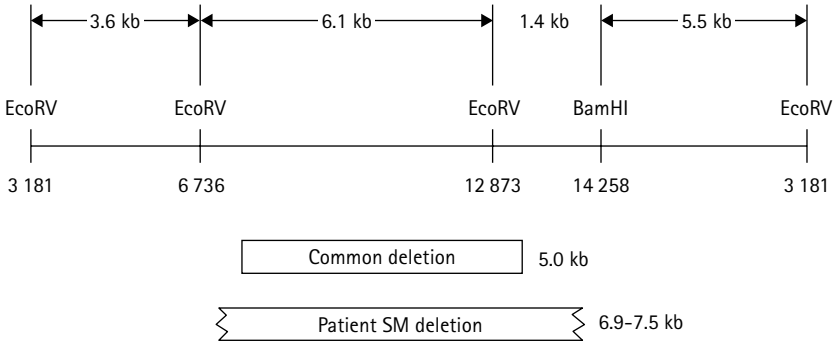

Figure 54.5 Linear representation of the common deletion and the deletion in a patient who presented first with 2-oxoadipic aciduria [15].

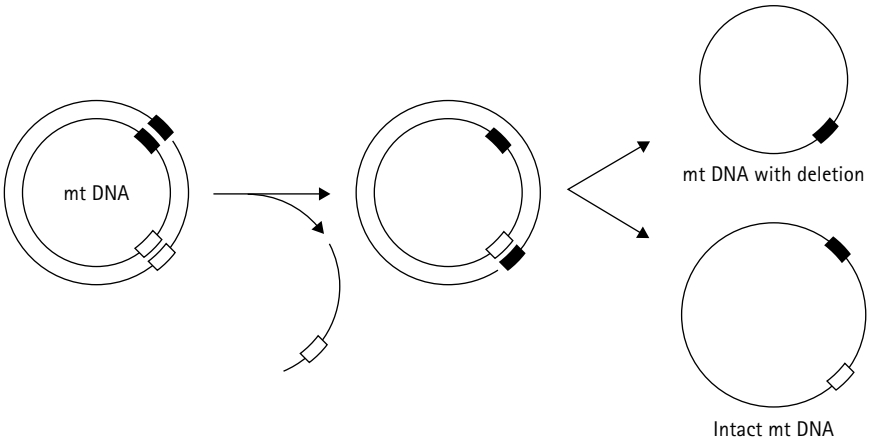

Figure 54.6 Slip replication model for the genesis of a deletion in mitochondrial DNA. Following the development of breaks, the smaller segment is removed and degraded.

between two areas of direct repeats could occur through slip-replication, in which, following a break at the first direct repeat the first repeat pairs with the second direct repeat (Figure 54.6).

In some patients, the abnormality in mitochondrial DNA is a duplication [33, 34]. In most instances, the clinical presentation is no different from that of patients with deletions. Some have had renal tubular acidosis or other renal tubular abnormality [22, 23]. The children reported [14] with hypoparathyroidism and deafness in Kearns–Sayre syndrome had duplications, as well as deletions. The deletions all spared four genes of complex I, including ND3 and ND6, as well as both genes of complex V, ATPase 8 and 6. The sizes of the duplications were inversely proportional to the sizes of the deletions. Homologous recombination, as well as slip replication, could be the mechanism of these rearrangements [35, 36].

Although it is unusual, maternally inherited mitochondrial rearrangement can be seen in this disease [37]. Identical deletions have been found in Kearns–Sayre, Pearson, and CPEO syndromes.

Profound deficiency of cerebral folate concentration, despite normal serum concentrations was found in a patient with a large deletion [38].

TREATMENT

Coenzyme Q10 may be of benefit. Treatment with 60–120 mg daily was reported [39] to be associated with decrease in modestly elevated levels of lactic and pyruvic acids and improvement in the prolongation of the PQ interval on the ECG, as well as ocular movements. The QRS complex did not change. Concentrations of folic acid and of carnitine may be reduced in plasma or muscle, and treatment with these agents may be useful. A vitamin B complex supplement is often prescribed. Two patients with cerebral folate deficiency were successfully treated with folic or folinic acid [7, 39]. In one [7] treatment with folinic acid at 2.5 mg/kg/day led to ambulation with ataxic gait in a patient who had lost the ability to walk. After one year of treatment, the 5-MTHF in the CSF was normal. Cranial MRI revealed improved myelination.

In the presence of complete A–V block, a cardiac pacemaker is usually required. Corrective eyeglasses may be helpful.

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Pearson syndrome

MAJOR PHENOTYPIC EXPRESSION

Sideroblastic anemia with vacuoles in marrow precursors, exocrine pancreatic insufficiency, hepatic dysfunction, small stature, mitochondrial myopathy, neurologic degeneration, lactic acidemia, and deletions in mitochondrial DNA.

INTRODUCTION

A syndrome was first described in 1979 by Pearson and colleagues [1] in which four unrelated patients had refractory sideroblastic anemia with variable neutropenia and thrombocytopenia, and clinical and pathologic evidence of pancreatic dysfunction. One of these patients later developed Kearns–Sayre syndrome [2]. In 1991, study of this patient by McShane and colleagues [3] revealed a 4.9-kb deletion in mitochondrial DNA. This 4977-bp deletion was located between nt 8488 and nt 13,460. This deletion was also that most commonly observed in patients with Kearns–Sayre syndrome. The same deletion (Figure 55.1) had been reported in 1988 by Rotig and colleagues in an infant with Pearson syndrome [4]. Rotig and colleagues [5] have since studied a larger series of nine patients with Pearson syndrome, including one of Pearson's original patients; five had the previously identified 4.9-kb deletion, and four had distinctly different deletions in the same area of the genome. A consistent feature was the occurrence of direct repeats at the boundaries of the deletions [6], providing a possible mechanism for recombinations. Rotig and her colleagues [7] have since found a patient in whom there was an insertion, as well as a deletion in the mitochondrial DNA. In all patients studied, there was heteroplasmy of normal and deleted mitochondrial genomes.

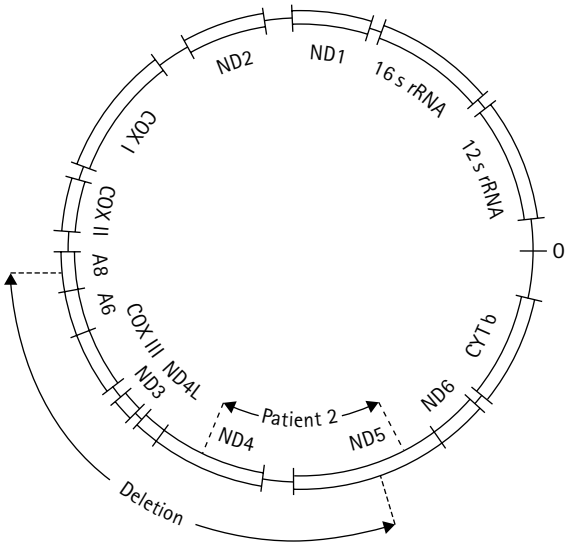

Figure 55.1 The mitochondrial genome and the deletion of 4.9 kb most commonly found in Pearson syndrome.

CLINICAL ABNORMALITIES

Patients with this syndrome (Figures 55.2–55.5) have severe transfusion-dependent anemia [1]. Onset is in the early weeks of life and pallor may be noted in the neonatal period. Anemia is macrocytic and aregenerative. Reticulocyte percentages are low. Hemoglobin F levels may be increased, and the free-erythrocyte protoporphyrin level may be increased [1]. Neutropenia and thrombocytopenia are variable. Either or both may begin concomitantly with the anemia or shortly thereafter, or pancytopenia may become progressively worse. In some, neutropenia may be episodic. Resistance to infection is impaired and death may occur in infancy, from infection such as *E. coli* sepsis. Death prior

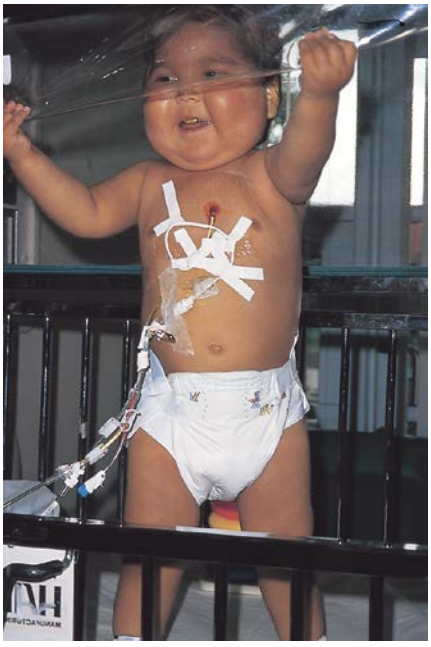

Figure 55.2 ES: A three-year-old boy with Pearson syndrome. He had failure to thrive, a renal Fanconi syndrome, anemia, and intestinal malabsorption. Renal biopsy revealed interstitial fibrosis and mitochondrial cytopathy. A Broviac port provided venous access for alimentation, as well as transfusion. He also had a gastrostomy.

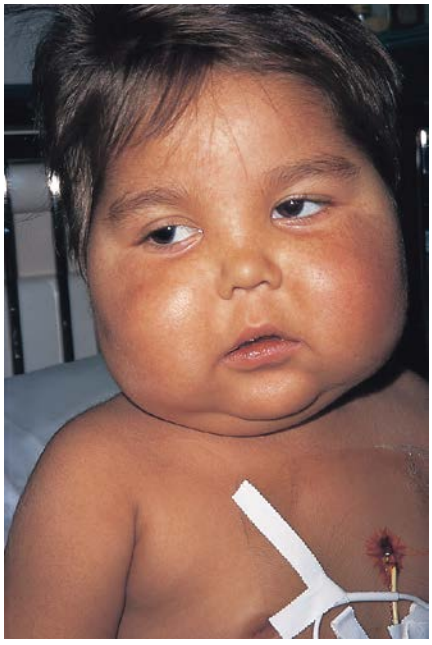

Figure 55.3 ES: The face was pudgy and hyperpigmented. Despite this Cushingoid appearance, there was no history of steroid treatment. He also had acanthosis nigricans.

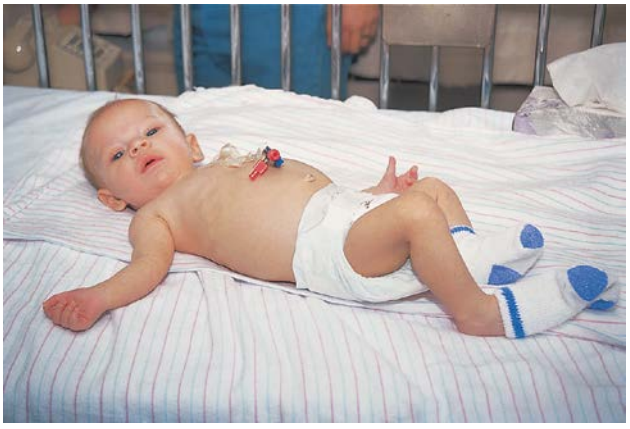

Figure 55.4 KK: A four-month-old patient with Pearson syndrome. He had neonatal onset pancytopenia requiring transfusion. Mitochondrial DNA deletion was documented. Illustrated is the failure to thrive, a consequence of his malabsorption, and the Port-a-Cath for transfusion.

to three years of age has been reported in 62 percent of patients [7, 8]. Neonatal death has been reported [9]. On the one hand, in patients surviving infancy, the anemia may disappear spontaneously, and the hemoglobin stabilizes as early as 11 months or two to three years of age. In such a patient, platelet counts may remain low. On the other hand, the anemia may first be evident at 13 months of age [10] with spontaneous recovery seven months later.

Bone marrow at the height of the anemia reveals increased cellularity, and there is striking vacuolization of both erythroid and myeloid precursors (Figures 55.6 and 55.7). The vacuoles are not those of fat, glycogen, or lysosomal material, for they do not stain with Giemsa, hematoxylin and eosin, Sudan black, or periodic acid Schiff (PAS). There are increased amounts of hemosiderin in the marrow and ringed sideroblasts (Figure 55.8). Electron microscopy revealed no limiting membranes on the vacuoles. The ringed sideroblast is a nucleated red cell with hemosiderin-laden mitochondria in a perinuclear arrangement. A variety of therapeutic modalities, such as prednisone, B_{12}, folate, and oxymethalone, were without effect.

Patients have varying degrees of pancreatic dysfunction. Some have had steatorrhea and malabsorption, but others do not. Tests of pancreatic abnormality have included decreased response of pancreatic enzymes and bicarbonate in duodenal aspirates to secretin-pancreozymin, and absence of stool or duodenal tryptic activity. Pancreatic fibrosis was documented in two patients at autopsy [1]. Another patient had chronic diarrhea but was not evaluated for pancreatic function. Another [11] had increased stool fat; this two-year-old girl also had diabetes and severe renal tubular acidosis. She had polyuria, proteinuria, glycosuria, phosphaturia, and generalized amino aciduria along with systemic acidosis, hypokalemia, and hypophosphatemia. Renal biopsy revealed tubular dilatation, degeneration of tubular epithelium and giant mitochondria in the proximal tubules. This patient also had hypotonia and had lost the ability to walk; there was muscle wasting and failure to grow. Computed tomography (CT) scan revealed cerebral

Figure 55.5 KK: He was pale, had lactic acidemia, and mild acidosis.

atrophy. Renal Fanconi syndrome has been observed in others [12]. Hypokalemia and hypercalciuria have been observed in other patients. One patient had renal cysts [13]. Two patients with Pearson syndrome developed insulin-dependent diabetes mellitus [14]. Dysphagia resulting from ophthalmoplegia weakness or incomplete opening of the upper esophageal sphincter or both may be observed [15, 16].

Similarities and differences between this syndrome and Schwachman syndrome, in which exocrine pancreatic insufficiency is associated with hematologic disease, have been considered [1, 14]. In Schwachman syndrome, the marrow abnormality leads to leukopenia, and the histology is of pancreatic fatty replacement. There is also bony

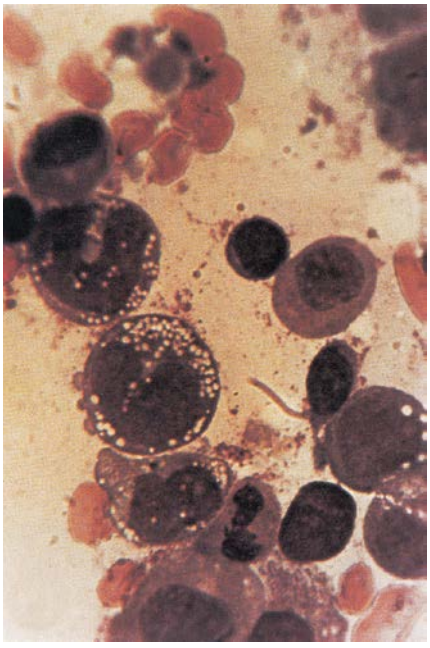

Figure 55.6 Bone marrow aspirate illustrating vacuolation of granulocyte precursors (magnification, ×1000). (Reproduced from the original paper of Pearson et al. [1] in the *Journal of Pediatrics* with permission from Elsevier.)

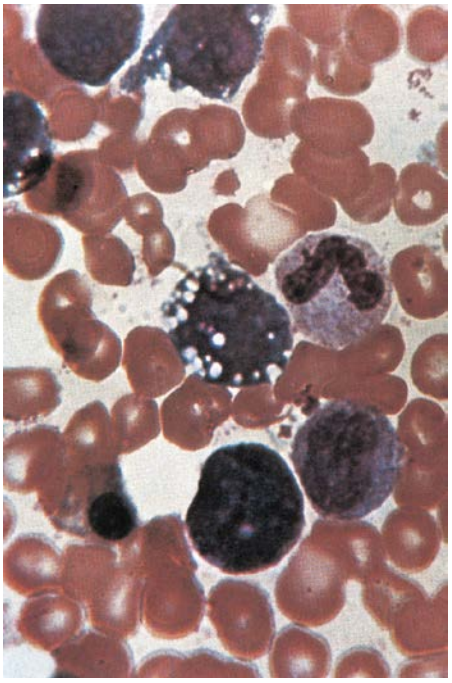

Figure 55.7 Bone marrow also reveals vacuolated erythrocyte precursors (magnification, ×1000). (Reproduced from the original paper of Pearson et al. [1] in the *Journal of Pediatrics* with permission from Elsevier.)

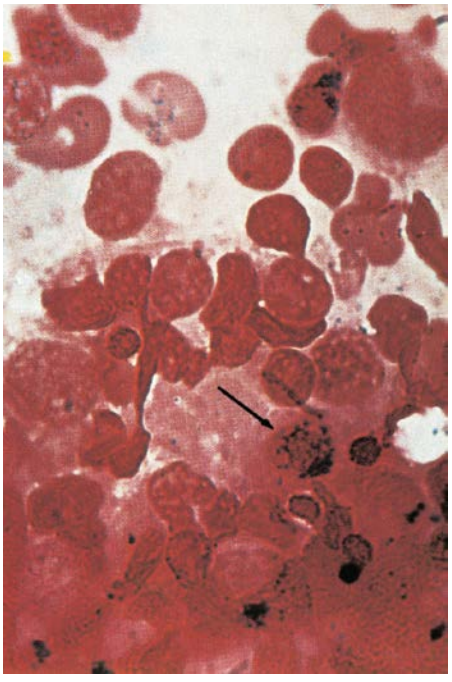

Figure 55.8 Ringed sideroblast is indicated by the arrow. There was hemosiderin throughout the marrow (magnification, ×1000, Prussian blue stain). (Reproduced from the original paper of Pearson et al. [1] in the *Journal of Pediatrics* with permission from Elsevier.)

metaphyseal dysplasia. In Pearson syndrome, vacuolation of marrow cells is distinctive; there has also been autopsy evidence of splenic atrophy [1]. In a patient with severe pancytopenia early in life, fibrosis of the thyroid was found at autopsy [17].

Hepatic dysfunction is another feature of Pearson syndrome. In the initial series, the first patient, who died at 26 months, had fatty infiltration of the liver; the second died of hepatic decompensation, and the liver at autopsy showed fat deposits, but no cirrhosis. A number of other patients have died in infancy of hepatic insufficiency [18]. Patients recovering from the marrow dysfunction may develop evidence of hepatic disease manifested by elevated transaminases, lactic dehydrogenase, and hypoprothrombinemia resistant to vitamin K. There may be jaundice.

Cataracts have been observed in Pearson syndrome [19]. Another patient had choroidal dystrophy [20] and hypogonadotropic hypogonadism.

Study of one of the original patients of Pearson *et al.* [1], at the age of 14 years, revealed a very different late-childhood/adolescent phenotype [2]. His hematologic disease began to improve spontaneously at seven months of age, and he had his last transfusion at 11 months, but macrocystosis and slightly decreased neutrophil and platelet counts persisted. Short stature was progressively evident; he was in the 25th percentile at six years and in the fifth at eight years. Over the next three years, growth velocity was 4 cm/year. His 24-hour integrated growth hormone level was judged to be low, and he was treated with growth hormone for four months. At 12 years, activity and mental alertness decreased, and he developed a tremor and a stammer. Speech and handwriting deteriorated, and his tremor became increasingly debilitating. Cognitive function was normal, and his IQ was 109. Deep tendon reflexes were brisk and there was unsustained clonus. He had a moderate lactic acidosis.

Patients with this disease may also have lactic acidemia in infancy [4, 9]. Some have died in episodes of metabolic acidosis. Magnetic resonance imaging (MRI) of the brain of such a boy revealed diffuse increase in T_2-weighted signal in the globus pallidus and pons and the white matter of the cerebral hemispheres, with periventricular sparing. This picture has been noted as having features of Kearns–Sayre syndrome [21]; it is more reminiscent of Leigh syndrome, at least on neuroimaging, and the dominating tremor of the clinical picture could be a different disease because of familial tremor in two generations. The fact that the affected family males were on the maternal side suggests that it is a consequence of the deletion. Other patients have had Leigh-type neuropathology [22].

The patient of McShane and colleagues [3], who presented in the neonatal period with Pearson-type anemia, later developed a typical Kearns–Sayre picture, with external ophthalmoplegia and pigmentary retinopathy, and mitochondrial myopathy on muscle biopsy, including ragged red fibers. The patient of Nelson *et al.* [23] had Kearns–Sayre syndrome with chronic progressive ophthalmoplegia and myopathy, having had sideroblastic anemia in infancy.

Progressive cardiac dysfunction, predominantly affecting the left ventricle, was reported [24] in a five-year-old boy. The authors suggested that this disease should be considered in patients with left ventricular dysfunction and suggestions of mitochondrial disease.

GENETICS AND PATHOGENESIS

The disease is caused by alterations in the mitochondrial genome (see Figure 55.1). Heteroplasmy has regularly been observed in affected individuals and the size of the deletion may vary considerably, although the location is always in the same area of the mitochondrial circular genome [3, 5]. Deletions have been identified in every tissue tested, including leukocytes, marrow cells, fibroblasts, and lymphoblasts of an original patient of Pearson [7]. The disorder is often described as sporadic and, in most families, there is no evidence of disease in the mother. However, identical deletions have been reported [10] in a son with Pearson syndrome and his mother with progressive external ophthalmoplegia. A daughter was unaffected. In another family [25], a mother and son had the identical 5355 deletion; he had typical Pearson syndrome, while she had progressive external ophthalmoplegia ptosis and weakness of pharyngeal facial, cervical, and limb muscles. Variability of this sort is consistent with the maternal inheritance pattern of mitochondrial mutations in which there is stochastic segregation of heteroplasmic DNA in the oocyte, and a bottleneck effect in which few of the very many mitochondrial DNAs are selected for the oocyte and new embryo. Certainly, a mother with clinical progressive external ophthalmoplegia is at risk for production of an infant with Pearson syndrome. A clinically normal mother could also have more than one offspring with this syndrome, especially if there were germ-line mosaicism. Similar clinical abnormalities with different deletions of mtDNA may be consistent with the fact that even small deletions include several tRNA genes and the transcript is not translated [26].

The most common mutation is a 4977 base-pair deletion (see Figure 55.1) from nucleotide 8482 to 13,460 [10]. This is also the most common deletion in Kearns–Sayre syndrome (Chapter 54) [27]. The deletion extends from the ND5 (NADH-CoQ reductase subunit 5) gene to the adenosine triphosphatase (ATPase) subunit 8 gene. There are two origins of mitochondrial DNA replication, with two different origins O_H and O_L for heavy and light chains, respectively, with replication of the former in a clockwise direction and the latter in the reverse. Since deletions usually spare O_H and O_L, there are two areas in which deletions occur most often, including all of those in Pearson syndrome; that is, in the larger O_H to O_L arc. The 4977-bp deletion is bounded by a pair of 13-bp direct repeats. This is a likely hot-spot for deletion. Rotig and colleagues [5]

found different types of direct repeats at the boundaries of five different deletions in the same area in nine patients. There was conservation in the 39 repeated sequences in the deletions and a certain homology between the nucleotide composition of six direct repeats and structures normally involved in replication of mitochondrial DNA and the processing of mitochondrial RNA. These repeats were particularly rich in pyrimidine nucleotides.

The deletions span coding segments for NADH dehydrogenase, cytochrome oxidase, and cytochrome b [28]. This would be expected to lead to disturbance in oxidative phosphorylation and would be consistent with the lactic acidemia observed. Abnormal redox was suggested [4] by a high lactate to pyruvate ratio of 30 (normal, below 20) and 3-hydroxybutyrate to acetoacetate ratio of 4 (normal, below 2). A larger 7767-bp deletion [23] led to deficient polarographic uptake of oxygen in the presence of NAD-linked substrates and enzymatic evidence of deficiency of all the complexes of the electron transport chain.

Two patients were reported [29] in whom deletions were found along with duplications and deletion dimers. One, considered to have a more attenuated phenotype because she died at ten years of age, had a linear duplication of 25 kb. The other girl, who died at 39 months with a lactic acidemia of 28 mmol/L, had a deletion dimer. Deletion dimers are combinations of two deleted fragments, while duplications are combinations of a normal and deleted fragment. It has been postulated that greater amounts of deletion dimers may correlate with greater severity of clinical manifestations [30]. Duplications may carry a better life expectancy [27], but this reflects experience with only the two patients studied. Duplications appear to be associated with increased recurrence risk as opposed to deletions only [31, 32]. The size and percentage of the deletions do not predict the clinical course or severity of disease [27]. Furthermore, there is no minimal region of deletion, the removal of which leads to Pearson or Kearns–Sayre syndrome. However, at least one tRNA must be deleted to cause either of these syndromes [33].

The spontaneous improvement of the anemia with time is of considerable interest. It is consistent with a concept of critical periods in the development of individual tissues. It has been postulated that there might be selective disadvantage with time of rapidly dividing hematopoietic cells containing a high proportion of deleted DNA. The opposite appears to occur in tissues like muscle and brain, where cells turn over more slowly; mutant DNA oxidative function diminishes, and mitochondrial encephalomyopathy develops. In these cells, deletions and duplications appear to have selective advantage over wild-type DNA, and, in contrast to mtDNA point mutations, they increase in proportion with time.

In a substantial cohort of patients reported from Italy, elevated levels of alanine in blood and fumaric acid in urine appeared useful indices for early diagnosis [34]. Alanine was elevated also in patients reported by Crippa *et al.* [35].

The random nature of partitioning of mitochondrial DNA during embryogenesis makes prenatal diagnosis unreliable with either amniocytes or chorionic villus cells. Rearrangements in mtDNA gradually disappear in cultured cells unless uridine is present in the culture medium.

In four patients with Pearson syndrome, 3-methylglutaconic acid excretion was elevated [36]. The authors suggested that the detection of this compound on organic acid analysis of the urine may be a useful marker for the disease.

TREATMENT

Refractory anemia requires repeated transfusion of blood. Erythropoietin is generally ineffective. Thrombocytopenia may require platelet transfusion. Pancreatic extract is useful in the management of the pancreatic insufficiency. It may also improve diarrhea and lead to weight gain [37].

Transplantation of hematologic stem cells corrected hematologic abnormalities, and metabolic acid acidosis [38]. The patient died of malignancy, but this approach to treatment was thought to be a reasonable option.

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56

The mitochondrial DNA depletion syndromes: mitochondrial DNA polymerase deficiency

MAJOR PHENOTYPIC EXPRESSION

Episodic neurologic deterioration and psychomotor regression beginning in the second year of life, with ataxia, encephalopathy, and failure to thrive, progressive to refractory seizures, cortical blindness, stroke-like episodes, acute fulminant hepatic failure, with micronodular cirrhosis, respiratory failure, and coma; fasting hypoglycemia, dicarboxylic aciduria, lactic acidemia, mitochondrial DNA depletion and deficient activity of intramitochondrial DNA polymerase γ.

INTRODUCTION

The first evidence for depletion of mitochondrial DNA in human disease was reported by Moraes and colleagues in 1991 [1]. Three hepatic (newborn, infantile, and toddler) and two nonhepatic, myopathic (infantile and toddler) forms are now recognized. The hepatic forms are more appropriately called hepatocerebral [2]. Both hepatic and myopathic phenotypes have been reported in the products of both consanguineous [3] and nonconsanguineous unions [4]. Each of the three hepatic forms is characterized by episodes of acute liver failure, fasting hypoglycemia, and mitochondrial DNA depletion. The two nonhepatic forms are characterized by nonepisodic myopathy, ragged red fibers, elevated serum creatine kinase (CK), and mitochondrial DNA depletion. An enzymatic diagnosis was established for the hepatic toddler form (Alper syndrome), in which mitochondria are deficient in the enzyme responsible for replicating mitochondrial DNA, DNA polymerase γ [5]. Mutations in the *POLG* gene have now been recognized [6, 7].

In the period since the initial description [1], more than 50 patients have been reported [8]. A diagnosis of mitochondrial DNA depletion may be suspected on the basis of fasting hypoglycemia and liver dysfunction characterized by elevations of gamma glutamyl transferase (GGT), often greater than alanine aminotransferase (ALT) and aspartate aminotransferase (AST), or by elevated CK and ragged red fibers. It is confirmed by quantitative analysis of mitochondrial DNA in biopsied tissue or, in the case of the hepatic toddler form, by demonstration of mitochondrial DNA polymerase deficiency. In the early infantile-onset hepatocerebral disease, a mutation in the deoxyguanosine kinase gene on chromosome 2p13 has been identified [9]. In addition to this single base deletion in the dGK (*DGUOK*) gene, missense mutations, duplications, and other deletions have been described [10]. In four families with myopathic disease, mutations were defined in the thymidine kinase gene (*TK2*) on chromosome 16q22 [11]. It is clear there is heterogeneity, because only about 10 percent of patients with similar phenotypes that were tested were found to have mutations in these two deoxynucleoside kinases [10, 12, 13]. Mitochondrial DNA depletion has also been found in patients with encephalomyopathy and defects in the succinyl-CoA synthetase gene (*SUCLA2*) [14]. In addition, mitochondrial DNA depletion has been found in patients with mutations in the twinkle helicase gene (*PEO1*) [15], the adenine nucleotide translocator gene (*ANT1*) [16], inner mitochondrial membrane protein gene (*MPV17*) [16–19], and the B subunit of ribonucleotide reductase

(RRM2B), as well as the thymidine phosphorylase gene (*TP*) in patients with mitochondrial neurogastrointestinal encephalomyelitis (MNGE). Patients with methylmalonic acidemia and mitochondrial DNA depletion have mutations in the succinate syntheses (*SUCLG1*) [14–19].

CLINICAL ABNORMALITIES

In the neonatal-onset hepatocerebral form of mitochondrial DNA depletion, the infants were small for gestational age (1.9–2.4 kg at term) and developed severe hypoglycemia (to 18 mg/dL, 1.0 mmol/L), and signs of severe liver dysfunction (prothrombin times of 23–30 seconds) in the first day of life. Hepatic size has been increased. Lactic acidemia, metabolic acidosis, and hyperbilirubinemia were inconsistent findings. Some patients had coagulopathy and increased α-fetoprotein. Mitochondrial electron transport studies showed global reductions in complexes I, II/III, and IV activity in biopsied liver, normal succinate dehydrogenase, and a two-fold elevation in carnitine palmitoyl transferase II. Skeletal muscle electron transport activities were normal. Death followed progressive hepatic failure between one and three months of life. Neurologic abnormalities included hypotonia, failure to develop, and horizontal nystagmus [3]. Histopathology of the liver revealed micronodular cirrhosis, cholestasis, glycogen-laden hepatocytes, microvesicular steatosis, and accumulation of iron. Some had giant cell formation. Electron microscopy revealed marked proliferation of pleomorphic mitochondria. In the liver, the levels of mitochondrial DNA were significantly depleted (7 percent of normal). Levels of mitochondrial DNA in muscle, kidney, and cultured fibroblasts were normal.

The infantile hepatocerebral form of mitochondrial DNA depletion is characterized by normal intrauterine growth, birth, and delivery [20, 21]. Nevertheless, it may not be different from the early infantile form. The first hint of trouble may be vomiting or feeding difficulty as early as the first month of life. Death may occur as early as seven months. Hypotonia and fasting hypoglycemia often prompt admission to hospital by two to three months of age. One patient was nine weeks old upon admission [21] and was found to have hepatic dysfunction characterized by elevations in GGT (353 IU/L) greater than AST (188 IU/L) and ALT (123 IU/L); total bilirubin was 5.9 mg/dL (2.6 mg/dL conjugated, 3.3 mg/dL unconjugated). Lactate in the blood was 7.16 mmol/L, and pyruvate was 0.23 mmol/L, yielding an elevated lactate to pyruvate ratio of 31 (normal $\leq$20), consistent with disturbed redox function resulting from a defect in mitochondrial electron transport. The serum concentration of bicarbonate was normal. Cerebrospinal fluid (CSF) concentration of lactate was 5.6 mmol/L. A monitored fast revealed hypoketotic hypoglycemia with 3-hydroxybutyric acid of 0.28 mmol/L and acetoacetate of 0.07 mmol/L. Glucose challenge raised the fasting blood lactate from 3.1 to 6.2 mmol/L. The patient was treated with a low fat (30 percent calories) diet, carnitine, riboflavin, and thiamine, but there was no significant change in blood lactate. Hypotonia persisted and gross motor development was poor. No other neurologic abnormalities were observed. Hypoglycemia became progressively worse with age, requiring feedings every 2–3 hours. The patient died at seven months of fulminant hepatic failure and coagulopathy. Light microscopy of the liver revealed steatosis. Electron microscopy revealed numerous mitochondria in which cristae were diminished in number or absent. Biochemical studies of liver mitochondrial electron transport complexes I, II/III, and IV revealed global reduction. Succinate dehydrogenase activity was normal. In skeletal muscle, only complex I activity was reduced. Quantitative studies of mitochondrial DNA showed normal levels in kidney, brain, and heart. Skeletal muscle mitochondrial DNA was 50 percent of normal. Mitochondrial DNA in the liver was depleted to 7 percent of normal.

The toddler form of hepatocerebral mitochondrial DNA depletion (Alper syndrome) [5] is associated with an enzymatic deficiency in intramitochondrial levels of DNA polymerase γ, the enzyme responsible for replicating mitochondrial DNA. Figure 56.1 illustrates an affected child at two years of age. This child had an older brother who had died at the age of 21 months during a second acute episode of hypoglycemia, status epilepticus, and acute hepatic failure associated with a 'Reye-like syndrome' that followed a febrile illness.

The toddler form of mitochondrial DNA depletion is characterized by normal intrauterine growth, birth, and delivery. Growth, fine motor, gross motor, cognitive, and language development were all normal in the first year of life. At 19 months of age, the patient depicted in Figure 56.1 had

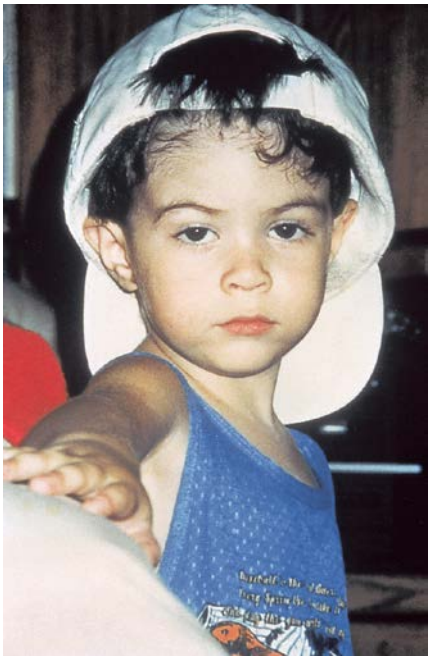

Figure 56.1 BW: At two years of age, with the hepatic toddler form of mitochondrial DNA depletion, 18 months before his death.

an episode of acute truncal ataxia, vomiting, hypoglycemia (34 mg/dL), associated with hypertonia and encephalopathy following a febrile diarrheal illness. The acute hypertonia gradually resolved to mild hypotonia. He recovered from almost all other deficits, leaving only mild residual truncal ataxia. After a second similar episode at 22 months and evidence of expressive language delay, he was evaluated for a possible disorder of fatty acid oxidation. The patient developed hypoglycemia (33 mg/dL) after 15 hours of a monitored fast. Blood acetoacetate was 0.44 mmol/L and 3-hydroxybutyrate was 3.8 mmol/L at the time of hypoglycemia, reflecting intact ketogenesis for age. Urinary organic acids after the fast showed only mild elevations in adipic (70 mmol/mol creatinine), suberic (36 mmol/mol), and sebacic (18 mmol/mol) dicarboxylic acids, and a trans-cinnamoyl glycine of 55 mmol/mol. Both long- and medium-chain triglyceride loads resulted in elevated excretion of urinary trans-cinnamoyl glycine (69 and 94 mmol/mol creatinine, respectively) and 3-hydroxydicarboxylic acids. No abnormalities in plasma amino acids were detected. Plasma free carnitine was reduced to 13.6 μmol/L. Urine carnitine was normal. ALT and AST were slightly elevated at 73 and 124 IU/L, respectively. Lactate in the blood was 2.3 mmol/L. Magnetic resonance imaging (MRI) of the brain was normal. The patient was treated with carnitine, cornstarch, and a low-fat diet.

The patient had six more episodes of decompensation over the following two years associated with febrile illnesses. Neurologic manifestations included truncal ataxia, erratic nystagmoid eye movements, focal myoclonic seizures, progressive failure to thrive, and stroke-like episodes. By the age of 38 months, he was unable to walk. At 41 months, he contracted a rotavirus infection that was associated with focal, left-sided epilepsia partialis continua (EPC), transient left hemiparesis, and cortical blindness. Liver enzymes started rising significantly at this time; the GGT was 987, AST 228, and ALT 288 IU/L. Bilirubin was normal. He died at the age of 42 months in liver failure and coma after a six-week terminal illness; lactic acid concentrations were up to 15 mmol/L.

Autopsy revealed advanced micronodular cirrhosis with regenerative nodules (Figure 56.2A). Electron microscopy of the liver revealed marked variation in mitochondrial content and morphology in neighboring hepatocytes. Some liver cells showed significant mitochondrial proliferation with a preponderance of tightly packed cristae and occasional mitochondria with concentric cristae (Figure 56.2B), while other liver cells had apparently normal mitochondria. Microvesicular fat and bile duct proliferation was noted throughout the liver. Skeletal muscle showed mild fiber size variation and mild increase in lipid staining. Electron microscopy of skeletal muscle showed mitochondrial proliferation with numerous pleomorphic forms (Figure 56.3). In the brain, neuropathologic examination of the frontal cortex showed marked neuronal loss and astrogliosis, and the appearance of Alzheimer type II glia. Subcortical white matter was normal. The primary visual cortex showed gliosis in layers II, III, and V, and perisomal and perivascular vacuolization (Figure 56.4A). Sections through the optic tracts and chiasm showed prominent spongiform vacuolization; however, luxol fast-blue staining

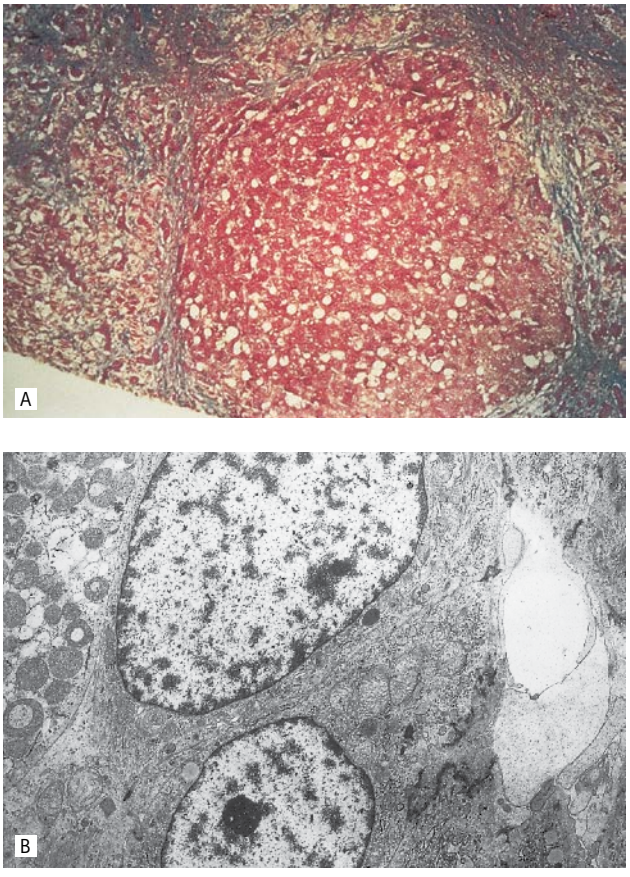

Figure 56.2 Hepatic histology. (A) Gomori-Trichome. Micronodular cirrhosis, regenerative nodules, microvesicular fat. (B) Electron microscopy. Marked cell-to-cell variation. Mitochondrial proliferation with abnormally packed lamellar and crescentic cristae in some cells, adjoining other cells with normal mityochrondrial numbers and cristae.

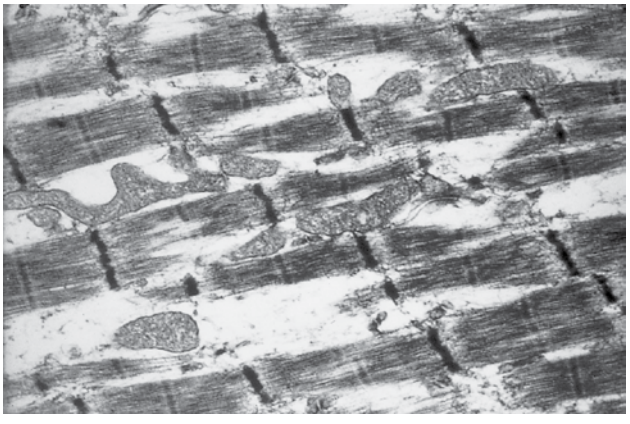

Figure 56.3 Electron microscopy of skeletal muscle. Mitochondrial proliferation and pleomorphic appearance. There were disordered fibers and mildy increased lipid.

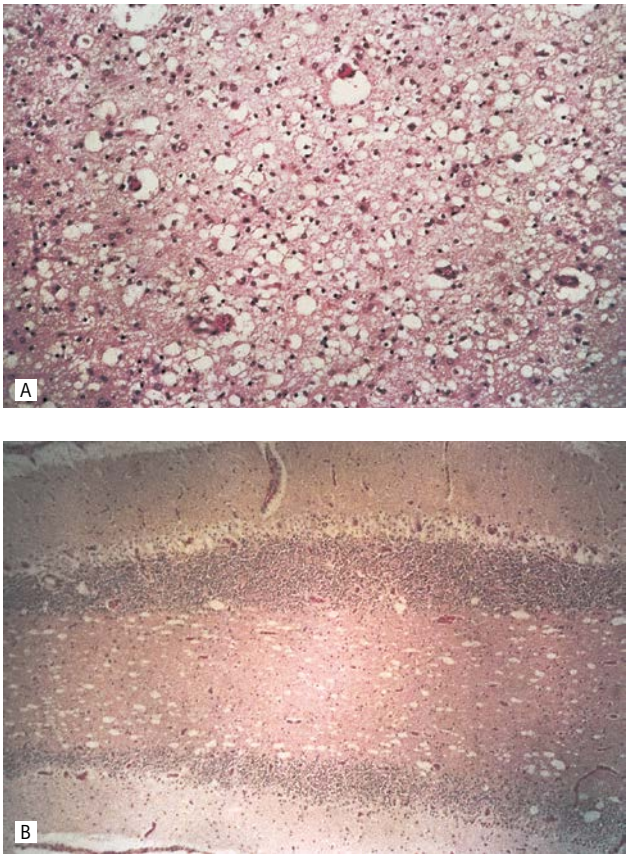

Figure 56.4 Neuropathology. (A) Occipital visual cortex. Fibrous gliosis in layers II, III, and V, with perisomal and perivascular vacuolization. Luxol fast-blue staining of these regions revealed normal myelination. (B) Cerebellum. Total loss of Purkinje cells, prominent gliosis, and sparing of the granular layer. The interfolial white matter displayed prominent spongy vacuolization. There was also focal sclerosis in the cerebellar vermis (not shown).

of these regions revealed normal myelination. The head of the caudate also showed gliosis. The cerebellar cortex showed a total loss of Purkinje cells, prominent Bergman gliosis and sparing of the granular layer (Figure 56.4B). The interfolial white matter displayed prominent spongy vacuolization. Sections through the vermis showed focal cerebellar sclerosis. The spinal cord also showed spongy vacuolization and gliosis affecting the anterior and posterior spinocerebellar tracts in the lateral columns.

Biochemical studies of the electron transport chain from skeletal muscle showed global reduction in the activity of complexes I, II/III, and IV. Mitochondrial DNA quantification revealed levels in skeletal muscle that were 30 percent of normal. Assay of purified mitochondria from skeletal muscle and liver revealed a complete absence of activity of mitochondrial DNA polymerase γ.

The nonhepatic infantile form of mitochondrial DNA depletion is characterized by normal intrauterine growth, birth, and delivery [1, 4]. Hypotonia, poor feeding, failure to thrive, and difficulty handling oropharyngeal secretions are noted in the first month of life. Hospital admission is usually prompted by poor motor development, vomiting, dehydration, or respiratory distress by the age of two to four months. At this time, the serum CK may exceed 1000 IU/L. Urinary organic acids are normal. Lactic acidemia may be present during periods of metabolic decompensation but is not a consistent feature. MRI of the brain reveals delayed myelination. Focal status epilepticus is a late feature. Muscle biopsy reveals abundant ragged red fibers, increased lipid droplets by Oil Red-O staining, and increased glycogen by PAS staining. Histochemical staining for cytochrome oxidase (COX) is absent in fibers. Biochemical studies of electron transport complexes I, II/III, and IV reveal global reduction. Muscle mitochondrial DNA is 2 to 8 percent of normal.

A fatal neonatal outcome was reported [22] in a patient with myopathic mitochondrial DNA depletion. He developed cyanosis, a weak cry, and generalized hypotonia immediately after birth. Spontaneous movements were diminished, as were reflexes. Ultrasonography of the brain showed periventricular hyperechogenicity and dilated lateral ventricles. EEG was abnormal. There was bilateral renal pyelectasis. There was metabolic acidosis (pH 6.99), lactic acidemia (21 mmol/L), and hyperalaninemia (1.226 mmol/L). He died at 36 hours. Activities of electron transport chain enzymes were markedly reduced in muscle, while in the liver there was only a mild reduction of complex I. There was a severe depletion of mitochondrial DNA in muscle while that of the liver was normal.

The nonhepatic toddler form of mitochondrial DNA depletion, which may not be different from the infantile form, is also characterized by normal intrauterine growth, birth, and delivery [1, 4]. In the first year of life, there may be frequent bouts of pneumonia, but without neurologic deficits. Cognitive and motor development are normal in the first year. Between 12 and 16 months, there may be increased stumbling or complete loss of motor milestones. Hyperlordosis and a waddling gait may be present. Patients with DNA depletion in muscle tend to have elevated CK. Serum CK is 500–2000 IU/L. Muscle biopsy reveals type I fiber predominance, abundant ragged red fibers, and a complete absence of COX activity by cytochemical staining. Patients may stop walking by the age of two years and be unable to sit unassisted by two and a half years. Neurodegeneration is progressive to death by respiratory failure in two to four years. Neuropathologic examination of cerebrum, cerebellum, brainstem, and spinal cord has failed to reveal abnormalities. Muscle mitochondrial DNA is 17–34 percent of normal.

Enlarging experience with mutations in POLG1 suggests that there is a spectrum of clinical phenotypes with presentations from the neonatal period to late adulthood [23]. Severe encephalopathy and hepatic failure represent one extreme. At the other end, are patients with autosomal dominant progressive external ophthalmoplegia (adPEO,

MIM 157640) and multiple mitochondrial DNA deletions. Others with multiple deletions have adult-onset cerebellar ataxia. Other dominant kindreds have Parkinson syndrome and premature ovarian failure. Patients with mutations in RRM1B had severe hypotonia, nephrocalcinosis and renal tubulopathy, hyporegenerative anemia, and congenital defects [18].

A pharmocogenetic–environmental interaction is susceptibility to hepatic reaction to valproate. In 50 percent of patients with abnormal infantile liver function, the hepatic disease developed within weeks of starting treatment with sodium valproate.

Most adults with POLG1 mutations developed progressive external ophthalmoplegia (PEO) and myopathy, often in association with ataxia and axonal peripheral neuropathy. Cardiomyopathy occurred in two patients. The majority of severe childhood presentations were in boys.

Two unrelated patients with unusual clinical and biochemical phenotype were reported by Yano and colleagues [24]. Both displayed developmental delay and hypotonia. One had cerebral cortical atrophy and she died in severe metabolic acidosis associated with pneumonia. Both had elevated plasma concentrations of glycine and methylmalonic aciduria (423–520 mmol/mol creatinine). Lactic acid was elevated in blood and urine. Activities of enzymes of the electron transport chain were variably reduced in muscle. The amounts of mitochondrial DNA in muscle were moderately reduced. One of these patients has since been found to have a mutation in the α unit of the succinyl-CoA synthesis gene *SUCLG1*.

Mitochondrial DNA depletion and mutations in mitochondrial DNA POLG have now been reported in Leigh syndrome [25].

Among patients with electron transport defects, the enzymatic deficiency may be expressed in the liver, even in patients with no sign of hepatic disease [26]. In a series of 31 patients with hepatic enzymatic deficiency, the deficiency was exclusively in the liver. These observations provide an argument for liver biopsy in patients who appear to have mitochondrial disease.

Mitochondrial DNA depletion has usually been documented by Southern blot analysis, but the method requires a large amount of DNA, is time-consuming, and susceptible to a number of artifacts. Recent experience with real-time quantitative polymerase chain reaction (PCR) was reported to be more efficient and to have higher sensitivity [27]. An algorithmic approach to the diagnosis of deficiencies of mitochondrial polymerase γ has been developed.

GENETICS AND PATHOGENESIS

All forms of mitochondrial DNA depletion are transmitted as autosomal recessive disorders [1–3, 26–28].

Molecular defects compatible with the clinical features of these syndromes could include abnormalities in the temporally regulated, tissue-specific expression of the mitochondrial DNA polymerase γ itself, or of one of the other essential components of the mitochondrial DNA replisome (Figure 56.5). One described defect is in the tissue-specific expression of the mitochondrial transcription factor A (mtTFA) required for production of RNA primers [29]. The cloning of the human mitochondrial DNA polymerase [30] has facilitated molecular dissection. The patient shown in Figure 56.2 had virtually a complete absence of mitochondrial DNA polymerase γ in liver and skeletal muscle.

Human DNA polymerase γ has been characterized as a reverse transcriptase [31, 32]. Two unrelated children with Alpers syndrome were found to have a homozygous mutation in exon 17 of the POLG locus that led to a glu873 stop. In addition, each was heterozygous for the G1681A mutation in exon 7 that led to an Ala467Thr substitution in the linker region of the protein [6].

The deoxynucleoside kinase defects, deoxyguanosine kinase and thymidine kinase [9, 10], indicate that balanced

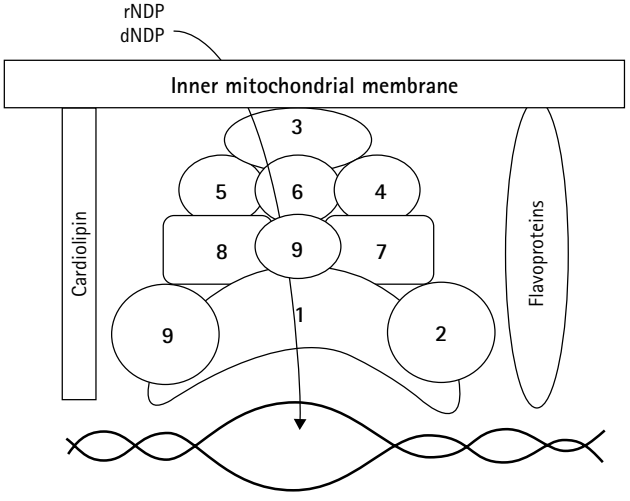

Figure 56.5 Enzymatic components of the mitochrondrial DNA replisome: nuclear contributions to mitochondrial DNA replication. All proteins involved in replication are transported to mitochrondria via chaperonins (cytoplasmic transport proteins). RNA and DNA are scrolled through the membrane-fixed replisome (comprising about 20 different proteins). Diagram is a model; geometry and composition of the replisome are not yet established. Nuclear gene products known to participate in mitochondrial DNA replication and repair include: 1, DNA Pol γ ($\alpha_2\beta_2$); 2, RNAse H (removes primer RNAs); 3, ribonucleotide reductase; 4, nucleoside diphosphate kinase; 5, dihydrofolate reductase; 6, thymidylate synthetase; 7, thymidine kinase; 8, topoisomerase I; 9, DNA ligase I; 10, DNA helicase; 11, ssDNA binding protein; 12, MTFA; 13, RNAse MRP; 14, cytosine deaminase; 15, endonuclease G; 16, mTERF (mitochondrial termination (actor)); 17, TAS-A-BP (termination sequence binding protein); 18, uracil N-DNA glycosylase (UDG, UNG); 19, dUTPase; 20, mitochondrial RNA polymerase; 21, dUMP/dCMO kinase.

pools of nucleotides in mitochondria are requisites for mitochondrial DNA replication. These defects provide an additional mechanism for mitochondrial DNA depletion [8]. The single-base deletion in the *dGK* gene causes a frameshift and leads to an undetectable enzyme protein [9]. Mutations in this gene and the missense mutations in the *TK2* gene accounted for only 10 percent of the patients with mitochondrial DNA depletion tested [10, 12, 13], so there had to be other causes of this syndrome. In a series of 32 patients with mitochondrial DNA depletion, a molecular cause was defined in 60 percent of patients with myopathy and 80 percent of those with hepatocerebral presentations [19].

Other candidate defects lie in the tissue-specific expression of chaperonins required for accurate trafficking of nuclear gene products, such as the polymerase components of the replisome (Figure 56.5), and components of the respiratory chain into mitochondria.

The mitochondrial genome is a circular double-stranded molecule containing 16,569 nucleotides and coding for 37 genes, including ribosomal RNA and 22 transfer RNAs. Each mitochondrion contains between two and ten copies of the genome.

Pathogenesis follows from mitochondrial DNA depletion. Since 13 protein subunits of complexes I, III, and IV and ATPase of the electron transport chain are encoded by mitochondrial DNA, its depletion would affect the activity of oxidative phosphorylation. The prominent hepatic abnormalities may be explained by the dramatic postnatal developmental changes and mitochondrial adaptation that occur in this organ in the first few months and years of life [33]. Postnatal adaptive changes in skeletal muscle mitochondria occur only later [34]. The existence of nonhepatic (more encephalomyopathic) forms of mitochondrial DNA depletion may reflect early disturbances in the myogenic program that result in either destabilization of the muscle cell membrane, or physical muscle cell breakdown with measurable increases in CK from muscle. Liver, brain, and muscle all "learn" or undergo adaptive metabolic changes that are shaped by encounters with the postnatal environment. High ratios of NADH to NAD^+ have been found in the severe deficiencies of complexes I, III, and IV in a patient with mitochondrial DNA depletion [21] and this should decrease mitochondrial ß oxidation and provide a mechanism for impaired fatty acid oxidation [35, 36].

Acquired mitochondrial DNA depletion syndromes have recently been described in adults as a complication of the treatment of HIV-1 and hepatitis B virus infections with the reverse transcriptase inhibitors azidothymidine (AZT) [37] and fluoriodoarauracil (FIAU) [38]. These nucleoside analogs are potent inhibitors of the mitochondrial DNA polymerase γ. In experimental animals, the mitochondrial DNA-depleting myopathy produced by AZT is reversible upon discontinuation of the drug. The delayed liver toxicity of FIAU was apparent only after patients had received the drug for 6–8 weeks and was not reversible upon discontinuation of the drug. Clinical trials of FIAU were suspended in 1994 when six patients died in acute liver failure. Encephalopathy and ataxia were frequent findings in patients with AZT or FIAU toxicity, but causality was difficult to establish because of coexisting disease. Secondary mitochondrial DNA depletion with near-fatal metabolic acidosis and hepatic failure has now been reported [39] in an infant with an HIV infection treated with AZT, didanosine, and melfinavir. A 79 percent reduction in mitochondrial DNA of muscle reverted to normal after discontinuation of antiviral therapy.

It is interesting to note that the toxicity syndrome of FIAU more closely resembles the hepatic forms of mitochondrial DNA depletion, while the toxicity syndrome of AZT more closely resembles the nonhepatic (more encephalomyopathic) forms. The unexpected biochemical action of these reverse transcriptase inhibitors *in vivo* and the striking clinical overlap between the inborn and acquired forms of mitochondrial DNA depletion stand as clear reminders that our knowledge of developmentally regulated and organ-specific mitochondrial DNA metabolism and replication is far from complete.

TREATMENT

Avoidance of fasting is an important element in management of the hepatic forms of mitochondrial DNA depletion. Uncooked cornstarch at 1 g/kg three times a day, or at least at bedtime, is useful in preventing hypoglycemia. Carnitine (60–100 mg/kg per day) and cofactor therapy including a multivitamin supplemented with coenzyme Q10 (4 mg/kg per day), riboflavin at 50–100 mg twice a day, and niacin at 10–25 mg twice a day appear to be helpful. A diet low in fat (30 percent of calories) appears prudent.

Myoclonic seizures in the hepatic toddler form of the disease have been difficult to control. Trials of clonazepam or amantidine (5 mg/kg per day) should be considered early, if seizures are not controlled by first-line anticonvulsants or ACTH. Valproic acid should be specifically avoided. Lactic acidemia is often a late complication of mitochondrial DNA depletion and may be reduced by treatment with dichloroacetic acid (50 mg/kg per day), but clinical efficiency has not been observed. Thiamine or biotin supplementation has not been successful in reducing lactic acid concentrations.

The multisystem abnormalities in the two later-onset hepatic forms of mitochondrial DNA depletion argue against the potential benefit of liver transplantation.

Treatment of the nonhepatic forms of mitochondrial DNA depletion also includes carnitine and cofactor therapy. Other supportive measures include good oropharyngeal secretion management, and early

gastrostomy tube placement with fundoplication to avoid aspiration and provide adequate nutrition. Management of the rare bouts of metabolic acidosis is with fluids and bicarbonate.

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PART 6

DISORDERS OF CARBOHYDRATE METABOLISM

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57

Galactosemia

MAJOR PHENOTYPIC EXPRESSION

Hepatomegaly, jaundice, vomiting, failure to thrive, cataracts, impaired mental development, renal Fanconi syndrome, urinary reducing substance, deficiency of galactose-1-phosphate uridyl transferase.

INTRODUCTION

Galactosemia is an inborn error of carbohydrate metabolism that results from deficiency of galactose-1-phosphate uridyl transferase (EC 2.7.7.12) (Figure 57.1). The disorder was first described in 1935 by Mason and Turner [1]. They found the reducing sugar in the urine and characterized it chemically as galactose. It is now clear that galactosuria may also occur in galactokinase deficiency, and in uridinediphosphate-4-epimerase deficiency. The enzyme deficiency was discovered by Isselbacher and colleagues [2]. The pathway of galactose metabolism had been worked out a few years earlier by Leloir and by Kalckar and their colleagues [3, 4]. The first step in the utilization is its conversion to galactose-1-phosphate (Gal-1-P) [5], which is catalyzed by galactokinase:

$$\text{Galactose} + \text{ADP} \xrightarrow{\text{galactokinase}} \text{Gal-1-P} + \text{ADP}$$

Gal-1-P is then converted to glucose-1-phosphate (G-1-P) in a series of two reactions in which uridine diphosphoglucose (UDPG) functions catalytically. The first of these is the uridyl transferase reaction (Figure 57.1), which is followed by the epimerase reaction in which the uridinediphosphogalactose (UDPGal) formed is converted to UDPG [6].

In developed countries, galactosemia is currently detected by programs of neonatal screening in which the transferase enzyme, or galactose content, is assayed in blood. Early diagnosis and compliance with dietary treatment obviate the classic manifestations of the disease. Nevertheless, we continue to learn from experience, as late complications are recognized in patients who have had early diagnosis and exemplary management. These have included abnormalities in language development [7, 8] and ovarian failure [9–11].

The gene has been assigned to the short arm of chromosome 9, at 9p13 [12]. In classic galactosemia, the classic mutation is a nucleotide change which leads to a p.Q188R change in the enzyme [13]. In patients with Duarte variant detected by newborn screening but not manifesting clinical illness, the mutation is expressed as p.N314D [13].

CLINICAL ABNORMALITIES

Manifestations of galactosemia [1, 14–16] usually appear within days of birth or of the initiation of milk feedings, and they increase in severity in the first months of life. Vomiting and jaundice may develop as early as a few days after milk feedings are begun. Vomiting has rarely been of sufficient severity to lead to surgery for a diagnosis of pyloric stenosis [17]. Anorexia, failure to gain weight or to increase in length, or even weight loss ensue. Hepatomegaly (Figures 57.2 and 57.3) is a constant finding on examination. Parenchymal damage to the liver is progressive to typical Laennec cirrhosis. Patients may have edema, ascites (Figure 57.3), hypoprothrombinemia, and bleeding. Splenomegaly may develop as portal pressure increases. If milk feedings are continued, the disease may be rapidly fatal.

Patients with galactosemia may present first with sepsis neonatorum. The organism is most commonly *Escherichia coli*. In fact, prior to the advent of neonatal

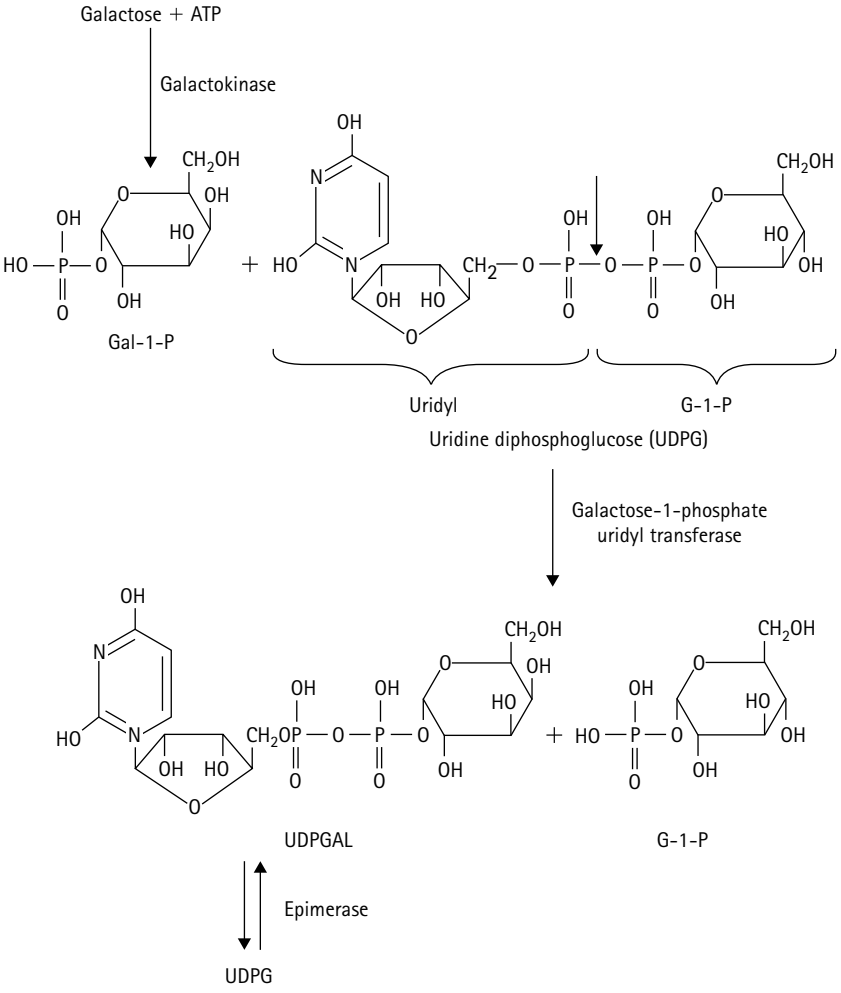

Figure 57.1 Galactose-1-phosphate uridyl transferase, the site of the enzyme defect in patients with galactosemia. The brackets indicate the uridyl and glucose-1-phosphate moieties of uridine diphosphate glucose (UDPG) which have split at the arrow in the uridyl transferase reaction, transferring the uridyl group from G-1-P of UDPG to galactose-1-phosphate (Gal-1-P) to form uridinediphosphogalactose (UDPGal).

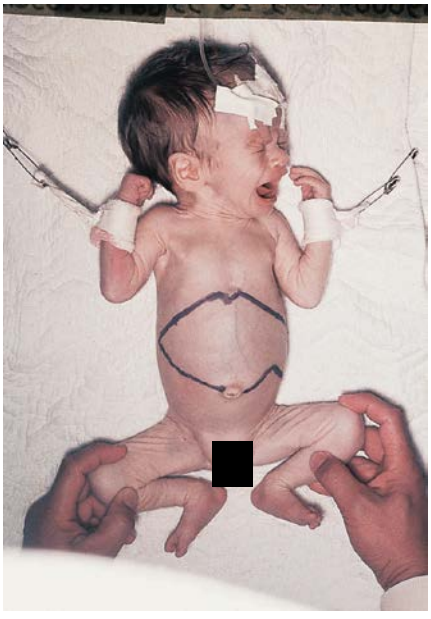

Figure 57.2 Classic presentation of the infant with galactosemia. Hepatomegaly is outlined below the upper line of the rib cage. Failure to thrive is evident in the virtual absence of subcutaneous fat and the folds of loose skin.

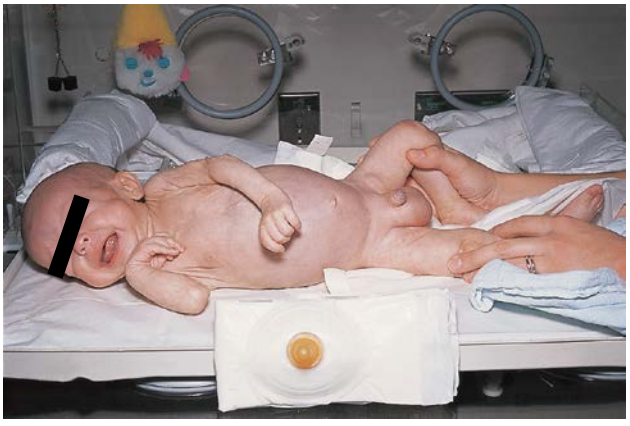

Figure 57.3 DS: An infant with galactosemia not diagnosed until 48 days of life. By this time, he had cataracts and failure to thrive. The abdomen was protuberant as a result of hepatomegaly and ascites. He also had edema of the legs and scrotum.

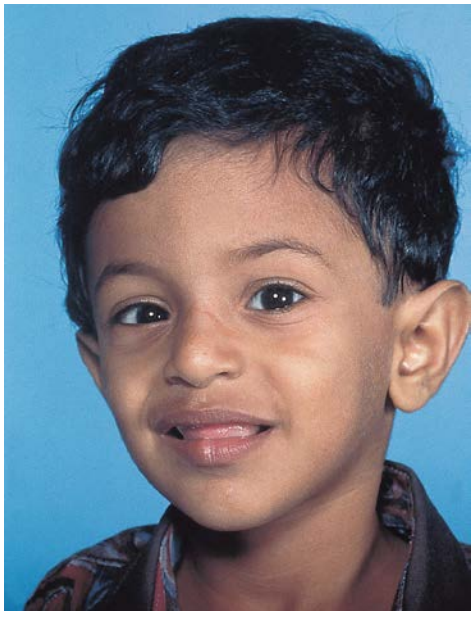

Figure 57.4 Saudi boy with galactosemia. Both lenses were removed because of dense cataracts. He usually wore thick glasses, but they were discarded for the photograph.

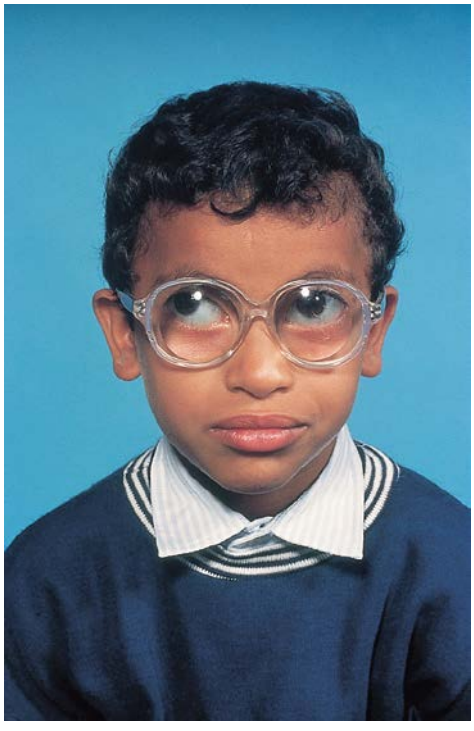

Figure 57.5 A: Another Saudi boy with galactosemia and the glasses he wore following surgery for cataracts. His diagnosis was made at one year, at which time he had hepatomegaly 8 cm below the costal margin, bilateral ankle clonus, and patchy white-matter abnormalities on computed tomography (CT), and was unable to sit or stand. Dietary treatment resolved the hepatomegaly. Developmental testing revealed borderline normal intelligence.

screening programs, the recommendation for the routine testing for galactosemia in all infants with sepsis led to most of the early diagnoses we encountered. A fulminant course of septicemia with early demise has been reported [18]. Complications of sepsis, such as osteomyelitis and meningitis, have also been observed. One patient developed gangrene of the toes bilaterally and of the dorsum of one foot [19]. Granulocyte function may be impaired [20, 21].

The development of lenticular cataracts is a characteristic feature of the disease and occurs in infants who have received milk for 3–4 weeks (Figures 57.4 and 57.5). Early cataracts may be visible by slit lamp examination, as early as after a few days.

Impaired mental development is an important manifestation of the disease. It is most severe in patients who are not diagnosed or treated until a number of months has elapsed. Untreated or poorly compliant patients are often hyperactive. In a series of 41 patients from before the advent of neonatal screening, three had severely impaired mental development, seven had IQ levels between 70 and 84, and 29 had IQs greater than 85 [22]. This experience, of course, reflects some siblings of patients in whom treatment from birth was possible. In another series of 44 [23], eight had IQ levels below 70; ten had levels from 71 to 89; and the rest had normal IQs, but lower than those of unaffected siblings. A relationship to compliance with diet was evident in an average IQ of 84 in 32 highly compliant patients and 77 in 22 poorly compliant patients [15].

Pseudotumor cerebri has been observed in a number of patients with galactosemia (Figure 57.6) [24]. It may be recognized by bulging of the anterior fontanel or

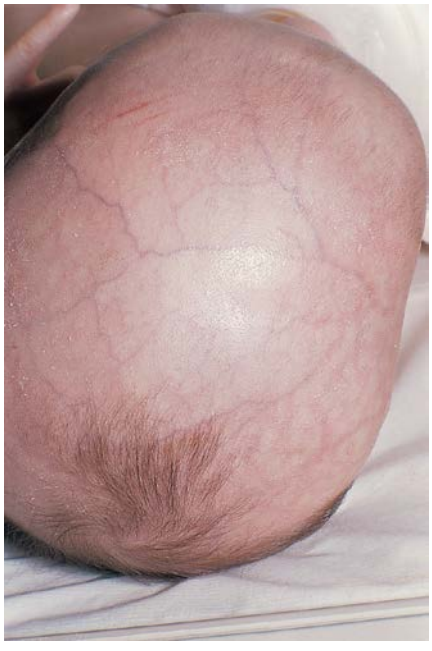

Figure 57.6 DS: Pseudotumor cerebri. The head circumference was increased and the fontanel bulging; the venous pattern was prominent.

by computed tomography (CT) scan [25] or magnetic resonance imaging (MRI) in which cerebral edema is evident. The occurrence of pseudotumor cerebri in patients with galactokinase deficiency [26] indicates that it is the accumulation of galactitol that causes this feature of the disease.

Renal abnormalities are usually first detected in the laboratory by the analysis of the blood and urine. Some patients have had frequency of urination. The picture is that of the renal Fanconi syndrome, in which there is renal tubular glycosuria, generalized aminoaciduria, and proteinuria, and systemically there is hyperchloremic acidosis. The glycosuria of the Fanconi syndrome may cause the galactosuria to be missed. In the past, the initial clinical suspicion of galactose has come from the presence of reducing substance in a sample of urine that tests negative for glucose with glucose oxidase, but once renal dysfunction develops, both tests would be positive. In any case, most clinical laboratories now test for urinary sugar with glucose oxidase. Tests for reducing substance have become the province of the biochemical genetics laboratory. At times of acute illness, there may be hypoglycemia. In young infants, hematologic examination may reveal an erythroblastotic picture.

In general, long-term follow-up study on galactosemic individuals [27] showed that when diagnosis is early and compliance with therapy is good, levels of patient IQ have been normal. Experience with differing times of initiation of therapy, including sibling pairs in whom therapy could be started on the first day of life, provided a trend that indicated the earlier the diagnosis, the higher the IQ. A number of children have had problems in school, so that the performance may not be as good as the IQ would suggest, but overall results have been excellent.

While dietary therapy for galactosemia has effectively eliminated the acute toxicity syndrome of classical galactosemia, long-term complications have become evident as significant problems, even under ideal conditions of management and patient compliance.

Ovarian failure has been recognized in female patients [9–11]. It may present as either primary or secondary amenorrhea with hypergonadotrophic hypogonadism. This is seen in 75–96 percent of female patients by the age of 30 years. The incidence of ovarian failure is unrelated to the age at diagnosis or the degree of dietary control. The mechanism remains enigmatic. Impaired oocyte maturation and accelerated atresia have both been reported. One patient had normal ovaries at laparoscopy at seven years of age and streak ovaries ten years later, suggesting a time-dependent effect. Pregnancies have occurred in female patients with classical galactosemia, although they are very rare. One patient, who successfully delivered, developed ovarian failure later. Many have low levels of estradiol and elevated levels of gonadotropins. Diminished or absent ovarian tissue may be revealed by ultrasonography. Evidence of hypergonadotropic hypogonadism has also been found in prepubertal girls [10]. The effect on the ovary is clearly a toxic one that takes a variable period of time to develop.

In female patients with hypogonadism, thyroid hormone levels were normal, but low concentrations of thyroxin have been reported in two galactosemic infants in whom levels of T_4 became rapidly normal when a galactose-free diet was instituted [28]. Testing for thyroid function in such an infant would be suggested by the presence of jaundice; the finding of a low T_4 might lead away from the diagnosis of galactosemia. This problem should be less frequently encountered where there are programs of neonatal screening, because currently, virtually all infants are tested for galactosemia and hypothyroidism before the development of symptoms.

The second major later complication of classical galactosemia is delayed speech and language [7, 8, 29, 30]. Onset of speech has been delayed, and there have been problems of articulation and word retrieval. Most children with galactosemia have delayed language development associated with a verbal dyspraxia, but it is often overcome with time. This complication also appears to be unrelated to the time of diagnosis or the level of compliance as assessed by erythrocyte levels of galactose-1-phosphate. Some of those individuals had never received milk, and exemplary galactose-1-phosphate concentrations had been maintained.

Cognitive development is the most important long-term issue in this disease. Impaired mental development is severe in patients who are diagnosed and treated late. Prior to the advent of neonatal screening, 11 of 85 patients had IQ levels below 70. An average IQ of 84 was seen in 32 highly compliant patients and 77 in 22 poorly compliant patients. Early information on the development of patients diagnosed early and compliant with therapy was optimistic [27]. By 1972, data from the largest experience in the United States suggested that such patients had normal levels of IQ, and it appeared that the earlier diagnosis, the higher the IQ.

However, more recent experience has led to a much more pessimistic prognosis. The results of a retrospective questionnaire survey of 298 patients from the United States and Europe on whom IQ data were available [30] indicated that 45 percent of those at least six years old were developmentally delayed. This survey provided the first evidence of a definite decline in IQ with age; furthermore, the decline in females was significantly greater than in males. In a more recent retrospective study of 134 galactosemic patients in Germany, there was also evidence of decline in IQ with age in that four of 34 patients less than six years of age had IQs less than 85, ten of 18 between seven and 12 years of age, and 20 of 24 older than 12 years had such levels. A best-fit regression line suggested a mean loss in cognitive performance of two IQ points per year; 40 points in 20 years. Of course, most of these patients, especially the older ones, antedated nationwide neonatal screening in Germany, and in the earlier international study, 270 patients had clinical symptoms prior to diagnosis and treatment. Data were not specifically set out in either study for patients diagnosed presymptomatically and managed carefully.

Nevertheless, decline with age in the earlier study was even shown in individuals tested at different ages. In addition, there was evidence in both studies of microcephaly and specific neurologic manifestations, such as progressive ataxia and tremor.

MRI of the brain has revealed a substantial number of patients with cerebral atrophy [31]. White matter abnormalities occurred in 95 percent (52 of 55) over one year of age and persisted in follow-up studies one to four years later. In addition, many patients, even with normal IQs, have had problems with behavior and school performance.

A curious syndrome of neurologic abnormality was reported [32] in siblings with galactosemia. Both had impaired mental development, hypotonia, and a coarse tremor. Ataxia developed, and neurologic tests of cerebellar function were abnormal. Dietary control of galactose intake was excellent and documented by determination of levels of Gal-1-P. These manifestations are reminiscent of chicks given lethal doses of galactose [33]. On the other hand, there is a possibility that the siblings each received two rare, recessive, possibly linked genes, even in the absence of consanguinity.

An interesting observation was the development of galactose toxicity despite continuation of a lactose-free diet in a homozygous woman, during lactation. The development of cataracts has been reported in lactating women, even heterozygotes [34, 35].

GENETICS AND PATHOGENESIS

Galactosemia is inherited as an autosomal recessive trait [36]. The enzyme defect in galactosemia is in the uridyl transferase enzyme (see Figure 57.1) [2]. The abnormality can be detected in the erythrocyte. Cord blood is a useful source for early diagnosis. The defect can also be detected in cultured fibroblasts and amniotic cells, leukocytes, and liver [37]. The other enzymes of galactose metabolism are normal.

The enzyme is a dimer in which each identical subunit has a molecular weight of 44 kDa [38]. In patients with classic galactosemia, the activity of the enzyme is virtually completely absent [37, 39]. In heterozygotes, the levels are intermediate between patients and normals [39]. The Beutler assay [40], in which the glucose-1-phosphate product is converted to glucose-6-phosphate and the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) formed is determined fluorimetrically, has been widely adopted for purposes of neonatal screening [41].

The demonstration of the enzyme defect in galactosemia was the first evidence for human variation at the Gal-1-P uridyl transferase locus. There has since been evidence of abundant variation, and the first to be discovered was the Duarte variant [42]. This enzyme has a distinct electrophoretic pattern of rapid migration [43], and its activity is about 50 percent of the normal enzyme. It produces no clinical manifestations. A number of other electrophoretic variants has been described. The Los Angeles variant, a rapidly moving enzyme with three bands, has normal or greater than normal activity [44]. The others all have less than normal activity, and in some, there may be clinical manifestations. A black variant may have no erythrocytic activity, but has about 10 percent of normal activity in liver and intestine [45]. These patients may have some neonatal symptoms. The Munster variant is associated with a classic galactosemic picture [46]. Gel electrophoresis [47] and isoelectric focusing [48, 49] have been employed to distinguish the variants, but they are being supplanted by molecular methods of determination of mutation.

It has become apparent that compounds in which an individual is heterozygous for two distinct variants are relatively frequent occurrences. Compounds in which one gene is the galactosemic (G) and one the Duarte (D) variant have been the most commonly encountered [50], especially in programs of neonatal screening for galactosemia [50, 51]. Most individuals with this phenotype have no clinical manifestations, but transient jaundice, lethargy, and hepatomegaly have been reported [51] in an infant whose mother had sepsis prior to the delivery; and others have displayed biochemical evidence of accumulation of galactose and Gal-1-P in the blood. Transferase levels may be very low early in life [51] and galactose tolerance tests have yielded evidence of diminished ability to metabolize ingested galactose. Doubt has recently been expressed that the N314D leads to reduced enzyme activity [52] indicating that a 5′4bp deletion leads to the enzymatic phenotype.

Polymorphism complicates the determination of heterozygozity, but family study may elucidate the problem. The mean enzyme activity for heterozygotes for the galactosemia variant (GN) approximates half that of normal (N) individuals, and this is the level observed in Duarte homozygotes (DD). However, since heterozygotes for the Duarte variant have about 75 percent of normal activity, the study of parents should clarify the issue. Prenatal diagnosis has been carried out by assay of the enzyme in cultured amniocytes [53] and chorionic villus material, and by the direct measurement by gas chromatography-mass spectrometry (GCMS) of galactitol in the amniotic fluid [54].

Programs of neonatal screening for galactosemia have indicated a frequency of one in 50,000 [55]. The galactosemia–Duarte (GD) compound occurs in about one in 3000–4000 [55]. In one year's experience in California, the frequency of galactosemia was one in 123,000 and that of the compound was one in 38,000, while the three-year experience with the program yielded an incidence of galactosemia of one in 86,000. In Massachusetts, screening of six million neonates yielded a figure of one in 62,000.

In classic galactosemia and in the Duarte variant, there are immunoreactive transferase proteins (cross-reacting material [CRM]) and the size and structure of these proteins are similar to those of the normal enzyme [56].

The transferase gene has been localized to chromosome 9p13 [12, 57–59] and the cDNA has been cloned from

human fibroblasts [60]. The gene is small; 11 exons and 10 introns are found in 3.9 kb. A number of mutations have been identified (Table 57.1) [61–65]. In classic galactosemia, an A to G missense mutation codes for a change from glutamine at position 188 to arginine. In the Duarte variant, an A to G mutation in exon 10 has changed an asparagine to an aspartic acid at position 314 near the carboxy terminus. The most common African mutation is p.S135L. The A to G mutation in the p.Q188R variant introduces a site of cleavage by the restriction endonuclease *Hpa*II, which permits family studies and population screening. In addition to Q188R, p.K285N is also common in Caucasians; these two were found in more than 70 percent of alleles [13]. A 4-bp deletion in the 5' region of the *GALT* gene has been found to be linked to the Duarte allele and to yield reduced activity of the enzyme [66]. The DAT+314 as opposed to N is common in nonhuman species, such as chimpanzee, macaque, and mouse, suggesting that it is the ancestral gene [66].

An interesting biochemical phenotype was found in a family in which the proband had classical galactosemia [67]. He had inherited two mutations in cis from his father, p.N324D and E204K. From the mother, he received a mutation in the splice-site acceptor of intron C. Enzyme activity in the father was nearly normal. An asymptomatic sister had compound heterozygosity for three mutations, p.E293K–N324D/N324D. Her erythrocyte enzyme activity was normal. It was speculated that the codons for E203K and N314D led to intra-allelic complementation in cis, but in fact the result is in keeping with what was later established for the effect of the p.N314D Duarte mutation.

The ideal approach to diagnosis of galactosemia is through routine neonatal screening. A protocol for the screening and management of galactosemia is given in Table 57.2. The assay in the United States is for the activity of galactose-1-phosphate uridyltransferase in dried blood on filter paper. In some countries, the assay is for galactose, and this will also detect galactokinase deficiency. The test for galactose will also be positive in patients with congenital shunts from portal to systemic vessels [68]. A positive screening test is confirmed by quantification of activity in freshly obtained erythrocytes in the fluorimetric assay for NADPH formed along with glucose-6-phosphate from the glucose-1-phosphate product. In classic galactosemia, the activity approximates zero. Variants with greater activity than this can be elucidated by electrophoresis or by mutational analysis. It is important for clinicians to recognize the early clinical manifestation of galactosemia and its infectious complications, because some developed countries have given up neonatal screening for this disease, and even in screened infants, classical disease can develop before the results of screening are known. The screening assay is followed by quantification of activity in freshly obtained erythrocytes.

In California, testing for common mutations is carried out on the initial blood spots. Nine different mutations were detected in 73 samples flagged in the inborn screen [69].

In populations in which screening programs are not available, the diagnosis of infants with early symptoms is still initiated by the finding of galactose in urine. It is important to emphasize that testing of urine with glucose oxidase (Clinistix, Tes-tape) will not detect galactose; this is a strong argument for continued use of the older methods for the screening of urine for reducing substance (Benedict or Fehling test, Clinitest). We have also recognized galactosemia by finding galactose on GCMS of the urine sent for organic acid analysis. It is also true that the excretion of galactose in the urine depends on dietary intake of lactose; in an acutely ill patient admitted to hospital and treated with parenteral fluid therapy, the disease may not be recognizable by testing the urine because he or she has not received galactose for 24 to 48 hours.

Table 57.1 Mutations associated with galactosemia

Codon and amino acids substitution[a]	Nucleotide change	Phenotype	Prevalence in classic galactosemia		
			Caucasian %	Hispanic %	African American %
Q188R	CAG→CGG	G	62	58	12
V44M	GTG→ATG	G			
S135L	TCG→TTG	G	0		48
M142L	ATG→AAG	G			
R148W	CGG→TGG	G			
L195P	CTG→CGG	G			
R231H	CGT→CAT	G			
H319Q	CAC→CAA	G			
R333W	CGG→TGG	G			
N314D	AAC→GAC	Duarte	5.9 of non-galactosemia controls		

[a]Within galactose-1-phosphate uridyl transferase (GALT).

Table 57.2 Protocol for galactosemia

When newborn screening reports a low Gal-1-P uridyl transferase		Remove galactose/lactose from diet and prescribe a soy or other lactose/galactose-free formula. Breastfeeding is stopped
		Obtain blood for Gal-1-P and uridyl transferase mutations
Diagnosis of DD, DG, or GG	DD diagnosis	Return infant to normal diet, no follow up required
	DG diagnosis	Continue diet until infant is 8–12 months old
		Check Gal-1-P
		Begin full lactose/galactose diet for two weeks
		Recheck Gal-1-P
		If Gal-1-P is within normal range, continue unrestricted diet
		If Gal-1-P is elevated, then return to lactose/galactose restricted diet
	GG diagnosis	Continue lactose/galactose restricted diet
		Obtain blood for Gal-1-P, LFT, bilirubin, and albumin
		Refer to ophthalmologist
		Monitor monthly in metabolic clinic until four months old, checking Gal-1-P levels every two weeks
		If Gal-1-P levels indicate good control, change to monthly Gal-1-P levels and continue monthly clinic visits
		Second year of life: monitor every three months[a]
		2–5 years old: monitor every six months[a]
		6 years and older: monitor yearly[a]
		Evaluate language development at preschool age
		Evaluate ovarian function of teenage girls
		Encourage regular eye examinations
		Developmental assessment at 4, 8, 14, and 18 years

[a]May vary depending on control.

Characterization of the reducing substance found in a urine sample can be done in a number of ways. It is usually done by paper chromatography. Testing with paper infiltrated with galactose oxidase provides for an effective screening procedure [70]. Of course, sugar in urine of an infant who tests positive for reducing substance and negative for glucose oxidase is galactose, until proved otherwise, and indicates direct assay of the enzyme. In patients with normal activity of the uridyl transferase, assays are performed for galactokinase and epimerase.

The structure of galactose is identical to that of glucose, except for the position of the hydroxyl on carbon 4. Lactose, the principal sugar of mammalian milks, is the predominant dietary source of galactose. It is a disaccharide in which glucose and galactose are linked in an α-1,4-glucosidic bond in which an oxygen bridge connects carbon 1 of galactose and carbon 4 of glucose.

The pathogenesis of most of the clinical manifestations of galactosemia is the accumulation of Gal-1-P in tissues [71]. Among the best evidence for this is the observation that therapeutic measures that result in reduction of intracellular concentrations of Gal-1-P lead to prevention or disappearance of symptoms. It is clear that the manifestations of galactosemia do not occur in galactokinase deficiency, in which disease hepatic, renal, and cerebral damage is unknown. Thus, impaired mental development is not due to galactose itself. Cataracts and pseudotumor cerebri occur in patients with galactokinase deficiency [26, 72, 73], and these complications are due to galactitol. This byproduct of galactose accumulation occurs by its reduction at carbon-1 and is present in urine and tissues. In the lens, galactitol causes osmotic swelling and disruption of fibers. Osmotic swelling is also the mechanism of production of cerebral edema. In addition, cataracts that result from galactose treatment of rats are prevented by sorbinil, which inhibits aldose reductase, the enzyme that catalyzes the conversion of galactose to galactitol [74]. Galactitol has been demonstrated *in vivo* by proton magnetic resonance spectroscopy in the brain of an encephalopathic infant with galactosemia [75].

The pathogeneses of the later appearing dyspraxic speech and ovarian failure, as well as the potential loss of late cognitive function, are not clear. Low concentrations of UDPGal have been proposed as a mechanism [76]. Information on mutations has indicated that the Q188R/Q188R genotype is a significant predictor of developmental verbal dyspraxia in patients with good metabolic control as indicated by erythrocyte Gal-1-P levels less than 3.2 mg/dL [77].

The possibility of epigenetic effects has been proposed [78] in studies of four patients with quite different cognitive results. Three had the p.Q188R mutation and one who had severely impaired mental development had the usually milder p.S135L mutation. A gene expression profile identified aberrations in cell survival pathways, such as mitogen activated protein kinase (MAPK). Studies of glycosylation of N-linked glycoproteins revealed persistent aberrant glycosylation.

TREATMENT

The treatment for galactosemia is exclusion of galactose from the diet [79]. This is accomplished by the elimination of milk and its products. The mainstay of the diet for an infant is the substitution of casein hydrolysate or a soybean preparation for milk formulas. Education of the parents and of the child as he or she grows older on the galactose content of foods is important. A list of foods has been published that is useful in management [80]. The determination of the Gal-1-P content of erythrocytes is employed in monitoring adherence to the diet [81], and acceptable levels have been set at 4 mg/dL (150 mmol/L). When this is not available, the serum bilirubin and the transaminase levels tend to be employed.

Experience with early treatment supports the concept that effective treatment instituted in the first weeks of life can prevent most of the classic manifestations of the disease. At the other end of the scale, impaired mental development, once established, is irreversible, and if the diagnosis is delayed, some damage to the brain is inevitable. There may be abnormalities of visual perception, behavior problems, or convulsions. Cataracts are reversible if treatment is started within the first three months of life. Hepatic and renal manifestations of the disease are reversible. Late manifestations of language development and ovarian failure are not prevented by otherwise effective treatment. The results of treatment on long-term cognitive function are controversial, but it is clear that prognosis is not as good as it was once thought.

A recent report of long-term follow up of 28 patients treated for classic galactosemia showed mean below average function across a broad spectrum of cognitive measures [82]. However, there was a wide range, and some individuals had average or above average success.

Management of infants detected by newborn screening and found to have DG variants has been controversial, especially in women committed to breastfeeding their infants. In a Philadelphia report [83], 17 DG infants were treated with a low lactose diet for the first year and 11 received a regular diet. While there were significant differences in urine galactitol and red blood cell Gal-1-P throughout the first year, there was no variation in differences in developmental outcomes, including those of IQ and language, but the year of life for this assessment ranged from one to six years, and there were significant differences in adaptive scores.

Monitoring biochemical correlates of treatment in DG patients receiving a standard diet revealed erythrocytes galactose-1-phosphate concentrations in the reference range [84]. However, plasma concentrations of galactose and galactitol were about twice that of control. These elevations showed no relationship to developmental or clinical outcomes.

A recent reevaluation of dietary management of galactosemia [85] reviewed available literature and concluded that neonates with galactosemia should discontinue breast and cow's milk formula; soy formulas are acceptable, as the galactose containing compounds are not digestible, to free galactose; elemental formulas are used for premature infants; fruits and vegetables are permitted; specific hard cheeses, calcium and sodium caseinate may be included. Supplemental calcium and vitamin D should be provided, and serum 25-hydroxyvitamin monitored at least yearly.

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58

Glycogen storage diseases: introduction

INTRODUCTION

The glycogen storage diseases are characterized by the deposition of glycogen in tissue cells. They are a heterogeneous group with different etiologies and different clinical manifestations. The classic form of glycogen storage disease was first described by von Gierke in 1929 [1]. The glycogen from this original patient was isolated by Schönheimer [2] and was found not to differ from normal glycogen in optical rotation or in its composition of glucose residues. The resistance of this material to glycogenolysis by the patient's liver *in vitro* and its prompt degradation by normal liver led Schönheimer to the conclusion that an enzyme essential to glycogenolysis was missing. This appears to have been the first demonstration of the concept proposed by Garrod [3] that inborn errors of metabolism result from genetically determined deficiencies of single enzymes. The demonstration by Cori and Cori [4] of the virtual absence of the activity of glucose-6-phosphatase in livers of patients with classic von Gierke disease established the deficiency of a single enzymatic step in carbohydrate metabolism as the basis of this disease.

Glycogen is a branched, polydisperse molecule that has been recognized since the time of Claude Bernard as the storage form for carbohydrates in animal tissues. This polysaccharide is composed entirely of units of α-D-glucose and the units are joined together in 1,4 and 1,6 linkages (Figure 58.1) to form molecules with molecular weights in the vicinity of 1 to 4 million. The branched, tree-like structure (Figure 58.2) was worked out through the elegant studies of Cori and Cori and their colleagues, using stepwise enzymatic degradation [5–7]. A free reducing group occurs at only one point. The straight chains of glucose residues are linked together by α-1,4 bonds; branching occurs through 1,6 linkages. In normal human glycogens, 6–8 percent of

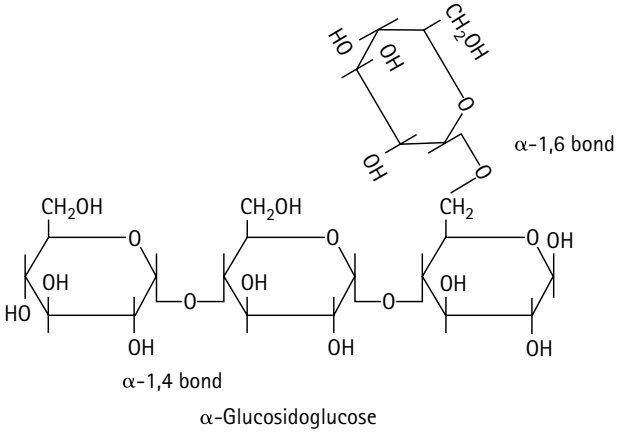

Figure 58.1 Portion of the glycogen molecule. The predominant structure is that of straight chains of glucose molecules in α1,4 linkage. The branch points in the structure are created by α-1,6 linkages. Cleavage of the 1,4 bonds is catalyzed by phosphorylase and cleavage of the 1,6 bonds by amylo-1,6-glucosidase, the debranching enzyme.

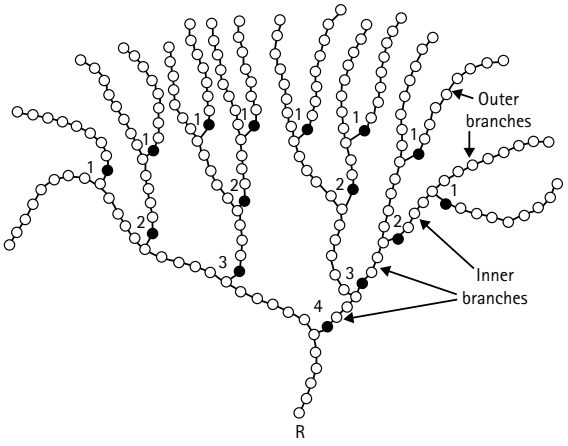

Figure 58.2 Structure of glycogen. The open circles represent glucose moieties in α-1,4 linkage and the black circles those in α-1,6 linkage at branch points. While four outer tiers of branch points are shown, glycogen has at least seven. R indicates the reducing end group. The outer branches terminate in nonreducing end groups. (Reproduced with permission from Cori [7].)

the glucose residues are joined to the rest of the molecule in α-1,6 linkage [8]. Glycogen contains seven tiers of branch points; the outer branches are terminated in nonreducing end groups [9].

The major pathway for the catabolism of glycogen is shown in Figure 61.1. The splitting of the 1,4 linkages in glycogen is catalyzed in the presence of inorganic phosphate by phosphorylase to yield glucose-1-phosphate [10]. The phosphorylase is activated by phosphorylation of serine, which is stimulated by glucagon and epinephrine [11]. Phosphorylase kinase catalyzes the phosphorylation and activation of phosphorylase [12]. Removal of phosphate from the enzyme is catalyzed by protein phosphatase and inhibits phosphorylase activity.

The phosphorolysis of glycogen catalyzed by phosphorylase splits off glucose units until the 1,6 branch points are approached. These branches are degraded down to a limit dextrin in which three glucose residues are attached in 1,4 linkage to the 1,6-linked glucose. The transfer of this trisaccharide to the end of another glycogen chain is catalyzed by the transferase activity of the debranching enzyme. Then, the exposed glucose at the branch point is cleaved by the same enzyme protein in which amylo-1,6-glucosidase activity is at a different catalytic site [13]. The product of the reaction is free glucose. The combined activity of phosphorylase and the debranching enzyme accomplish the complete degradation of glycogen.

Glycogen is stored in liver and muscle, and these are the tissues predominantly affected in the classic glycogenoses. Pompe disease, or glycogenosis II, is an exception because it is a lysosomal storage disease. Its major effects are cardiac and there is no problem with hepatic storage. The fact that enzymes, such as the phosphorylases, have different genetically determined enzymes in liver and muscle results in different diseases that have hepatic or myopathic clinical manifestations. Hepatic metabolism of glycogen is critical for glucose homeostasis; hepatic glycogenoses present classically with hypoglycemia. Muscle glycogen is used to make adenosinetriphosphate (ATP) for contraction; glycogenoses of muscle present with cramps, weakness, stiffness, or rhabdomyolysis.

Eight numbered types of glycogenosis result from specific defects in enzymes of glycogen catabolism (Table 58.1). They were given numbers in the chronological order of their description. The numbers seem less useful now that molecular defects have been identified, leading to multiple different forms of type I, as well as the disappearance of type VIII, which was once used for phosphorylase kinase deficiency. Nevertheless, these numbers are in such common use, they will be continued.

Ia. The classic form of glycogen storage disease originally described by von Gierke [1] is caused by a deficiency of glucose-6-phosphatase lb, (Chapter 59). Type I is divided into Ia, and Ib, the translocase deficiency that transports glucose-6-phosphate into microsomes.

Ib. Deficiency of glycose-6-phosphate translocase T1 deficiency. These patients have altered neutrophil function and susceptibility to gram positive bacterial infections.

Ic. Deficiency of glucose-6-phosphate translocase T2 which carries inorganic phosphate from microsomes to cytosol and pyrophosphates from cytosol into microsomes.

Table 58.1 Glycogen storage diseases

Enzyme deficiency	Glycogen structure	Organs	Synonyms	Type
Glucose-6-phosphatase	Normal	Liver, kidney	Hepatorenal glycogenosis	Ia
Glucose-6-phosphate transport protein	Normal	Liver, neutrophils	Translocase T_1 deficiency	Ib
Stabilizing protein	Normal	Liver	Regulatory protein deficiency	Iasp
Microsomal glucose transporter	Normal	Liver	GLUT7 deficiency	Id
α-1,4 Glucosidase	Normal	Cardiac	Cardiac glycogenosis, generalized glycogenosis	II
Amylo-1,6-glucosidase (debrancher)	Abnormal: very short outer branches	Liver, muscle	Limit dextrinosis	III
Amylo-(1,4→6) trans-glucosidase (brancher)	Abnormal: long, straight chains	Liver, muscle	Amylopectinosis	IV
Muscle phosphorylase	Normal	Muscle	Myophosphorylase deficiency	V
Liver phosphorylase	Normal	Liver	Hepatophosphorylase deficiency	VI
Muscle phosphofructokinase	Normal	Muscle, erythrocytes		VII
Phosphorylase[a]	Normal	Liver		IX
GLUT2	Normal	Liver	Glucose transporter	

[a] Autosomal recessive and X-linked phosphorylase kinase deficiency.

Id. Deficiency in the transporter that translates free glucose from microsomes to cytosol.
II. Type II, or Pompe disease, (Chapter 60) is a lysosomal storage disease which usually causes death in infancy from cardiomyopathy. In Type II glycogenosis infantile onset patients predominately have cardiac disease, while adult-onset patients predominantly have skeletal muscle myopathy.
III. Glycogenosis type III (Chapter 61) is the result of defective activity of the debrancher enzyme. It causes massive hepatomegaly in infancy and progressive myopathy in adults.
IV. Type IV, or Andersen disease, is a very different type of disorder in which defective activity of the debranching enzyme produces abnormal glycogen that appears to act as a foreign body and causes hepatic cirrhosis.
V. Type V, or McArdle disease, results from defective activity of phosphorylase in muscle. Symptoms are those of muscle cramps that limit exercise tolerance and myoglobinuria.
VI. Type VI, hepatic phosphorylase deficiency, [14], known as HERS disease, leads to a mild hepatic glycogen storage disease. Hepatomegaly may be the only clinical manifestation.
VII. Type VII glycogenosis, or Tarui disease, is clinically identical to McArdle disease, but the enzymatic defect is in the phosphofructokinase of muscle.
IX. Phosphorylase kinase deficiency is now known as type IX, but this enzyme is composed of four subunits coded by different genes, and hence the inheritance of its five different clinical subtypes is variously autosomal recessive and X-linked recessive [14]. The most common of these, affecting some three-quarters of type IX patients, is the X-linked recessive defect in the α-subunit [15]. The γ-subunit controls the catalytic center; the α β and δ subunits are regulatory. All but α are autosomal recessive. Patients with type IX disease often have elevations in transaminases. Those with defective activity in the A1 subunit have defective activity of phosphorylase kinase in liver, erythrocytes, and leukocytes. On the other hand, those with mutations in the A2 subunit, have normal kinase activity in erythrocytes and leukocytes, and sometimes even in liver.

Patients with type IX disease and with phosphorylase deficiency often present with isolated hepatomegaly (Figure 58.3), and are often first referred to the metabolic service after a liver biopsy has identified large amounts of glycogen. Most do not require treatment, and as adults, they have normal height and modest enlargement of the liver. Type IXa often displays an abnormal transminases hypercholesterolemia, hypertriglyceridemia, and fasting hyperketosis [15].

The Fanconi-Bickel syndrome combines a hepatic glycogen storage disease with a Fanconi syndrome pattern of renal tubular dysfunction [16]. Some have called it "type XI". Patients have failure to thrive and intolerance to galactose. These patients also resemble type I in that they have hyperlipidemia and hyperuricemia. The molecular detect is the liver type facilitated glucose/galactose transporter GLUT2.

Glycogen synthase deficiency (Table 58.2) is sometimes referred to as type 0 or GSD0, but this is a misnomer as there is no storage of glycogen. Patients have fasting hypoglycemia and ketosis; postprandially, they have elevation of lactic acid. They are often thought to have ketotic hypoglycemia. Glucose tolerance tests lead to substantial lactic acidemia. The enzyme activity can be tested only in liver. Current diagnosis is often made by a search for mutation.

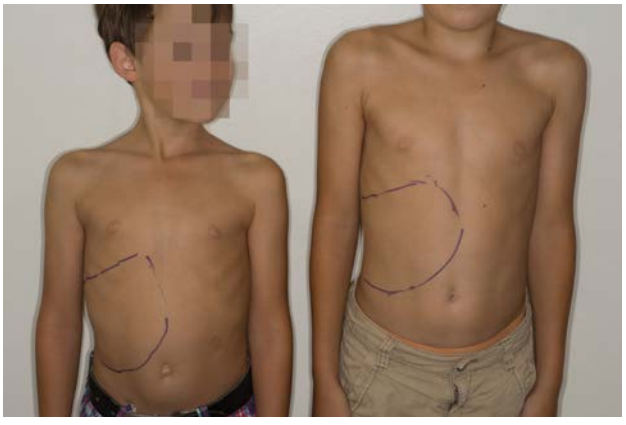

Figure 58.3 Two brothers with glycogenosis IX, they were short of statue and had prominent hepatomegaly. They were interesting in the sense that they never had any problems with glucose hemostasis. Both were good at sports.

Table 58.2 Glycogen synthase deficiency

Fasting	Fed	Glucose tolerance test
Hypoglycemia	Normoglycemia, hyperglycemia	Hyperglycemia
Increased acetoacetate, 3-hydroxybutyrate	Increased lactate after glucagon	Hyperlactic acidemia, free fatty acids, acetoacetate
Alanine and lactatrate low		3-Hydroxybutyrate normal
No response to glucagon		

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59

Glycogenosis type I – von Gierke disease

MAJOR PHENOTYPIC EXPRESSION

Hypoglycemia; massive hepatomegaly from storage of glycogen in the liver; short stature; prolonged bleeding time; ketosis, hyperlipidemia, lactic academia, and hyperuricemia; late complications of gout, hepatic adenomas, renal disease, and osteoporosis; impaired glycemic response to glucagon; and deficient activity of hepatic glucose-6-phosphatase.

INTRODUCTION

The classic form of glycogen storage disease originally described by von Gierke [1] is caused by a deficiency of glucose-6-phosphatase (Figure 59.1). It became apparent that there were subtypes of glycogenosis I and a considerably expanded glucose-6-phosphatase system when patients were studied who appeared to have von Gierke disease in which glucose-6-phosphatase activity in frozen liver was normal. The term glycogenosis type Ib was derived to distinguish these patients from those (Ia) in whom the activity of the enzyme is deficient [2, 3]. In 1978, Narisawa and colleagues [3] found defective glucose-6-phosphatase activity in fresh liver and restored activity by adding detergents; they suggested that the defect was in glucose-6-phosphate transport. The translocase defect was reported in 1980 by Lange and colleagues [4]. The gene (G6PT1) (SLC37A4) has been mapped to 11q23-24.2 [5]. Type Ic was recognized [5] on the basis of normal activity of glucose-6-phosphatase in detergent-disrupted microsomes, while activity in intact microsomes is defective for both glucose-6-phosphate and carbamylphosphate substrates, but molecular studies have indicated that both Ib and Ic are caused by mutations in translocase. Mutations in the G6PT1 gene have been found in the patients with type Ib or Ic disease [6]. Type Id with defective microsomal transport of glucose has not yet been observed clinically. A variant of type Ia is a result of the deficiency of the regulatory protein, designated Iasp, for stabilizing protein which has so far been reported in a single patient [7]; it is impossible to distinguish

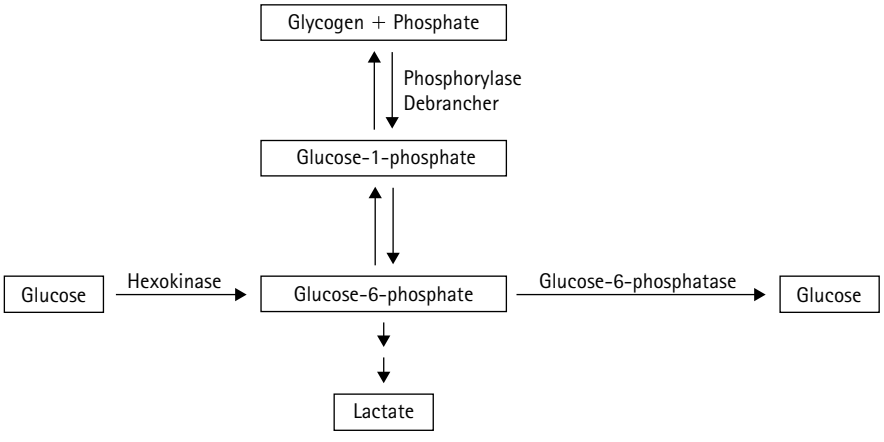

Figure 59.1 Glucose-6-phosphatase, the site of the defect in von Gierke disease.

this from deficiency of the catalytic subunit clinically, and difficult biochemically unless the entire stabilizing protein is missing.

The enzyme, glucose-6-phosphatase (G6PT1) OMIM 613742 [8] is expressed in the liver, but also in the kidney, pancreatic islets, and intestinal mucosa; and glycogen accumulates in all three organs. Clinical manifestations appear largely, if not entirely, consequences of the metabolic effects of the enzyme defect. Late effects, hepatic adenomas, and renal disease are of uncertain pathogenesis. The enzyme is situated in the endoplasmic reticulum, and this sets the stage for transport defects.

The gene for glucose-6-phosphatase has been cloned [9] and so has that of the translocase [10]. Mutations have been identified in the phosphatase [9], some of which are specific for certain ethnic groups, such as R83C and Q347X in Caucasians and G727T in Japanese [11]. Type 1a the phosphatase deficiency occurs in about 80 percent of patients; some 20 percent have 1b.

CLINICAL ABNORMALITIES

In classic type Ia glycogen storage disease, symptoms usually occur in the first months of life, and the disease may be recognized at birth. There may be neonatal hypoglycemia. Hepatomegaly is often present at birth [13] and progresses to huge enlargement of the liver without splenomegaly (Figures 59.2–59.7). The kidneys are also enlarged and may be visualized on roentgenography or may even be palpable. It is common in this condition for the liver to be palpable at the iliac crest in infancy and early childhood. The abdomen

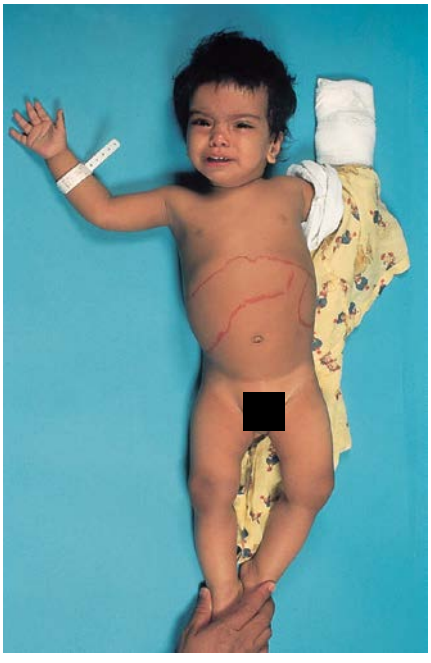

Figure 59.2 OH: An infant with glycogen storage disease type I. The face is somewhat chubby and the liver decidedly enlarged.

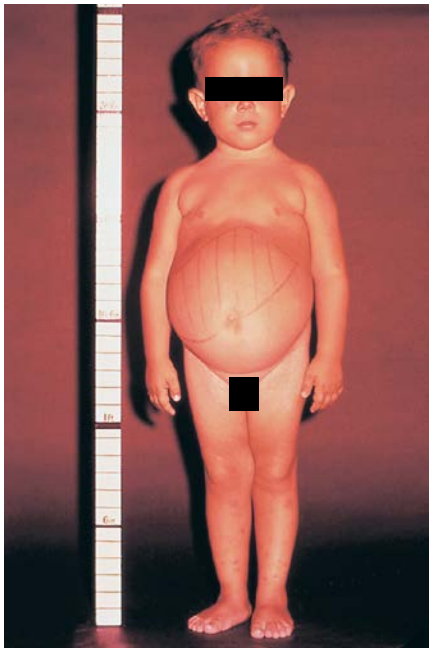

Figure 59.3 DA: A five-year-old boy with glucose-6-phosphate deficiency. He had massive hepatomegaly. There was some adiposity about the face.

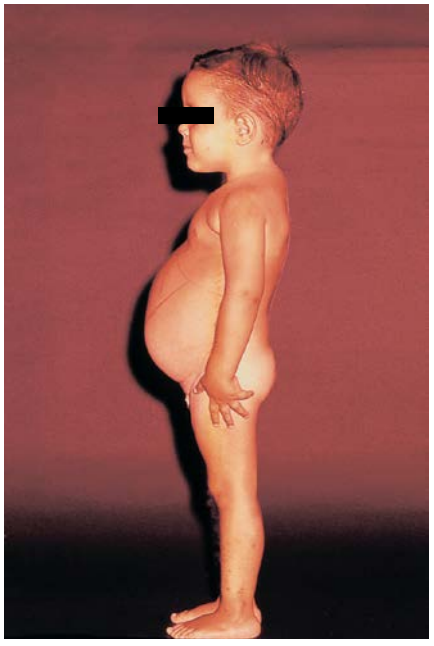

Figure 59.4 The abdominal enlargement is more impressive in the lateral view.

is protuberant, the posture lordotic (Figure 59.4), and the gait broad-based and rolling or swinging, all apparent consequences of the hepatomegaly. With time and growth, the abdomen tends to become less prominent.

Linear growth is usually impaired (Figure 59.6). The shortness of stature is symmetrical in that there is proportionate reduction in the length of the trunk and

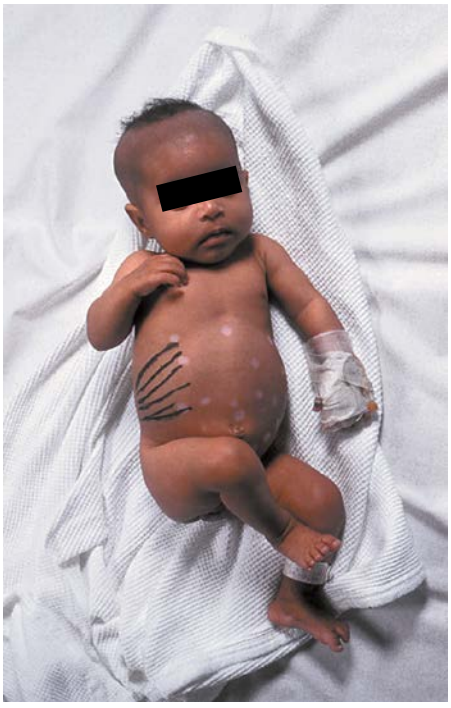

Figure 59.5 A six-month-old infant with glycogen storage disease (GSD) type I. He had the typical chubby cheeks, hepatic enlargement, and abdominal distention. He had hypoglycemia and acidosis. Diagnosis was made following liver biopsy. Depigmented spots were cautery marks, resulting from a common practice in the Middle East.

Figure 59.6 MS: A 60-year-old woman with glycogenosis type I. She was short. The scar represented recent surgical removal of a hepatoma. Abdominal girth was markedly reduced by the procedure.

Figure 59.7 MS: Facial appearance was still rounded. She appeared considerably younger than her chronological age.

extremities [14]. Adiposity, particularly about the cheeks, is the cause of the doll-like or cherubic appearance (Figures 59.3, 59.5, and 59.7). Musculature tends to be flabby and poorly developed and the legs appear thin.

Hypoglycemic symptoms tend to appear after three to four months, when the infant begins to sleep through the night. Nevertheless, symptomatic hypoglycemia may present in early infancy [15] or even in the neonatal period [16]. Manifestations of low blood sugar may include irritability, pallor, insomnia, feeding difficulties, and seizures. Episodes of hypoglycemia and metabolic acidosis in the neonatal period may be refractory to treatment [15], or so easily controlled that the diagnosis is not entertained until much later. Many infants present with vomiting or convulsions in the morning [17]. These symptoms are relieved by feeding or by the administration of glucose, and frequent feedings prevent their recurrence. With increase in the activity of the child at about one year of age, the frequency of hypoglycemic symptoms tends to increase. As in the case of any hypoglycemic syndrome, severe convulsions and permanent brain injury may be seen in patients with this disease.

In a series of 19 patients studied specifically for evidence of brain damage [18], abnormalities of the EE6 were found in four, only two of whom had seizures; some had abnormal evoked potentials, and all of these abnormalities correlated inversely with compliance with treatment. Abnormalities in magnetic resonance imaging (MRI) of the brain in seven correlated with neonatal hypoglycemia. None had impaired mental development. Children with this disease have an unusual degree of tolerance to hypoglycemia, often appearing quite well at levels of blood sugar at which convulsions

would ordinarily be expected [12], and the incidence of impaired mental development is not high. Possibly, this is a function of chronic hypoglycemia, where the rates of change of blood glucose are not very fast, but also parents and children become quite aware of the limits of tolerance for fasting. It could be relevant to utilization of lactic acid or ketones by the brain. Some patients are asymptomatic, discovered only by the presence of hepatomegaly on routine physical examination [15, 19], and one of our patients came to attention at surgery for an adenoma, with evidence of glycogen storage in the adjacent liver.

Bleeding may be a major clinical manifestation in patients with this disease. It may take the form of frequent nosebleeds in which there is considerable loss of blood. Abnormal hemostasis and persistent oozing may complicate surgery. These problems are thought to represent defective platelet function. Bleeding time and platelet adhesion are abnormal, and there is defective collagen and epinephrine-induced aggregation of platelets [20]. Many small superficial vessels may be visible under the thin-looking skin.

Intermittent episodes of diarrhea have been reported [14, 15, 21]. This has been attributed to malabsorption of glucose [22] but, in other studies, no evidence was found of malabsorption of monosaccharides, disaccharides, or fat [14, 15, 21]. Intestinal biopsy revealed no signs of inflammation and fecal α-1 antitrypsin was not increased [23]. One patient died from hemorrhagic pancreatitis [24].

Cutaneous xanthomas may develop over the buttocks, hips, elbows, and knees [25, 26]. Their occurrence is related to the elevated levels of triglycerides in the blood. Another consequence of hyperglycemia is the appearance of characteristic discrete, flat, yellowish retinal lesions [27] in the paramacular area; they do not adversely affect vision [18]. Patients with untreated hyperuricemia develop tophaceous gout [26, 28]. Most patients have osteoporosis and repeated spontaneous fractures have been seen in some patients [25]. Some of these patients have deficiency of vitamin D [29].

In addition to the hypoglycemia, a variety of abnormalities can be detected in the clinical chemistry laboratory. Lactic acidemia is a regular feature of the disease [30], and occasionally the level of pyruvate is increased. Marked hyperlipidemia and hypercholesterolemia are also features of the disease [12]. Concentrations of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) are high, and the apolipoproteins apoB, C, and E are high, while apoA and D are normal or low [31]. The hyperlipidemia leads not only to the formation of xanthomas, but also to large lipid-laden reticuloendothelial cells in the bone marrow. The plasma may be milky. It is important to recognize that the concentration of water in serum or plasma is markedly reduced in the presence of hyperlipidemia. In as much as electrolytes and other substances are only distributed in the water phase, extremely low values are recorded for the serum sodium and other constituents. A correction must be made for the increased quantity of serum solids and decreased serum water in order to avoid a mistaken diagnosis of hyponatremia.

Ketosis and ketonuria occur promptly with minimal degrees of fasting [30]. In fact, glucose-6-phosphatase deficiency is one of the few conditions in which ketones may be observed in the urine in the neonatal period. This and the lactic acidosis concomitantly may lead to metabolic acidosis. Despite the fact that ketosis has long been considered a characteristic of the disease, patients with this disease have been reported [32, 33] to be resistant to ketosis or to have decreased ketogenesis. Dicarboxylic aciduria has been observed [34] and this would be consistent with a suppression of the β-oxidation of fatty acids leading to ω-oxidation. Hyperuricemia is a regular concomitant of the disease [28]. This has been attributed to competition by lactic acid for renal tubular secretion and decreased clearance of uric acid has been observed. However, not all patients have reduced clearance of uric acid [35], and studies of uric acid production have provided evidence of increased purine synthesis in this disease [28, 36, 37]. A renal Fanconi syndrome of glycosuria, aminoaciduria, and phosphaturia has been reported in a number of patients with von Gierke disease [38, 39], but at least one of these patients [38] is now known to have Fanconi-Bickel disease. An unusual manifestation is elevation of biotinidase activity [40]. In two patients not previously diagnosed, biotinidase levels were 26 and 15 mmol/min/mL (normal mean, 7); in the second, it was this that led to the diagnosis.

The administration of epinephrine or glucagon fails to provoke the normal hyperglycemic response [41]. There may be some elevation of blood glucose after these agents, even in the virtual absence of glucose-6-phosphatase, for 6–8 percent of the glucose residues of glycogen are released as free-glucose as the product of the debranching enzyme. In response to glucagon or epinephrine, there is a marked increase in the concentration of lactic acid in the blood. In the absence of glucose-6-phosphatase, there is also a failure of the usual rise in blood glucose following the administration of galactose or fructose [15]. Similarly, following the intravenous (IV) administration of glycerol, elevation of blood glucose was less than normal and levels of lactic acid rose [42]. These abnormal responses to epinephrine, glucagon, fructose, glycerol, or galactose have, in the past, been used to diagnose the condition. Today, the glucagon test is employed for preliminary diagnosis and decision about candidacy for liver biopsy, and the definitive finding is the assay of the enzyme and the demonstration of the accumulation of glycogen in the cells of the liver (Figure 59.8). The existence of some common mutations means it is now possible to make a definitive diagnosis by mutational analysis and avoid biopsy of the liver.

Pathologic examination [1, 43] (Figure 59.8) reveals that the hepatocytes and renal tubular cells are swollen and clear as stained with hematoxylin and eosin; staining with Best's carmine reveals the stored material to be glycogen. There may also be extensive lipid storage in the liver. In fact, the lipid may be so prominent that the referring pathologic diagnosis may be lipid storage disease, such as Wolman disease (Chapter 94). In the neonatal period, the histology

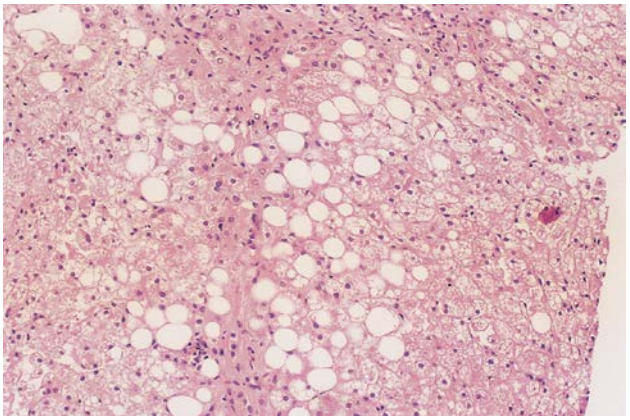

Figure 59.8 Biopsied liver of AK, a boy with glycogenosis type I. He clearly had storage of glycogen in distended hepatocytes, but there was also so much lipid that the initial pathologic diagnosis of Wolman disease was only discarded after the acid lipase was found to be normal, and he was referred to the metabolic service.

of the liver can be so normal that one is led away from the diagnosis [16].

Chemical analysis establishes that the material stored in the liver and kidneys is glycogen. Biopsy of the liver with demonstration of a glycogen content over 12 percent of the wet weight of the liver has been set out [30] as a criterion for the diagnosis of this disease. Schoenheimer [44] did not find quite that much glycogen, and most investigators would find values over 4 percent acceptable, but again, today the gold standard for diagnosis in biopsied liver is assay of the enzyme. The structure of the glycogen stored is normal [45].

A number of late complications have been observed, particularly as these patients reach adulthood. Hyperuricemia is present in infancy, but symptomatic gout occurs after adolescence. Pancreatitis [24, 46] appears to be a consequence of the hypertriglyceridemia. Adult stature is short and puberty is often delayed. Fertility is not affected, and both males and females have had children [47].

An appreciable problem in management has been the regular development of hepatic adenomas by the second or third decade [48]. These nodules are multiple, and they grow, sometimes to sizable tumors. They are usually benign, but transformations to malignant hepatocellular carcinomas have been recorded and may be fatal [48, 49]. Another complication is bleeding into the adenoma [50]. Patients should, therefore, be investigated at intervals for these nodules and, once present, their size and character should be followed. Scintigraphic scans have been recommended for this purpose [51], but ultrasonography and other forms of imaging have been useful in our hands.

A variety of renal complications has been observed. In addition to the renal Fanconi syndrome, patients have had distal renal tubular disease [52], amyloidosis [53], hypercalciuria, nephrocalcinosis, and calculi. Decrease in urinary citrate was found in 15 patients [54], reversing the normal increase with age, along with hypercalciuria. The authors suggested that treatment with citrate might be useful in preventing nephrocalcinosis and calculi. Glomerular hyperfiltration, increased renal plasma flow, and microalbuminuria [55] are followed over time by proteinuria, focal segmental glomerulosclerosis, and interstitial fibrosis [23, 56, 57]; followed in some patients by renal failure, leading to dialysis and transplantation.

Glycogenosis type Ib/c

Patients with glycogen storage disease type Ib [4] have the same type I clinical phenotype but, in addition, they have neutropenia and impaired neutrophil function (see Table 58.1) [58]. As a consequence, they have recurrent bacterial infections, inflammatory bowel disease, and ulceration of oral and intestinal mucosa [59–63]. Fecal α-1 antitrypsin is increased. Biopsy of the colon reveals inflammation [23]. Among 288 patients with type I glycogenosis, 57 had type Ib [62]. Neutropenia was found in 54. It is often documented in the first year of life, but may be noted first between six and nine years. It may be persistent but, more commonly, it is intermittent. Among 18 patients with neutropenia in whom neutrophil function was studied, it was abnormal in all. Apoptotic neutrophils were documented by increased activity of caspase, condensed nuclei, and perinuclear clusters of mitochondria in this disease, but not in other neutropenic disorders, such as Shwachman Diamond syndrome [64]. Perioral infections occurred in 37 patients, perianal infections in 27, and protracted diarrhea in 23. Inflammatory bowel disease was documented by colonoscopy or roentgenographic examination. Inflammatory bowel disease was not observed in the absence of neutropenia. Two Japanese patients were reported with no evidence of neutropenia and no recurrent bacterial infections [63]; otherwise, symptoms were typical of type Ib glycogenosis and there were mutations on both alleles of the gene.

GENETICS AND PATHOGENESIS

The disorder is inherited as an autosomal recessive trait. It has frequently been observed in siblings and as a concomitant of parental consanguinity [12, 25, 46, 65]. The distribution between the sexes is about equal. Ethnic differences in the severity of disease have been observed. For example, patients from Syria and Lebanon tend to have serious disease, while those from Saudi Arabia have had quite mild disease. Reduced activity of intestinal glucose-6-phosphatase has been found in parents of patients [66]. Prenatal diagnosis has been made by biopsy of the fetal liver and enzyme assay [67].

The molecular defect in glycogenosis I is absence of activity of the catalytic subunit of the glucose-6-phosphatase enzyme complex [2]. The enzyme is expressed normally in the liver, kidney, and in the β cells of pancreatic islets. The

diagnosis has generally been made by assay of biopsied liver. Absence or near absence of the enzyme has been required for the diagnosis [68, 69], because many enzyme activities, including this one, may be reduced in liver disease or in other storage diseases. The diagnosis can be made by needle biopsy, but sufficient complexity has been recognized that open biopsy and an adequate sample of tissue are preferred. Direct vision also protects against bleeding. Samples should also be fixed for light and electron microscopy. Care should be taken to avoid destruction of hepatic cellular membrane elements, and precautions for the handling of specimens prior to assay have been set out [5].

The active site of the enzyme is within the lumen of the endoplasmic reticulum [70]. Normal activity of the enzyme requires the activity of six different proteins or subunits in the enzyme complex [71, 72]. The discovery of these components and the elucidation of the function of the complex were the results of the study of patients with glycogen storage disease. The classic enzyme, or the catalytic subunit whose deficiency causes type Ia, is a 36.5-kDa protein [72] that catalyzes the hydrolysis of a number of phosphate compounds, including carbamylphosphate and pyrophosphate, as well as glucose-6-phosphate [73, 74]. A microsomal regulatory protein has been isolated as a 21-kDa stabilizing protein because it stabilizes the activity of the catalytic protein during purification [75]. It binds calcium and is essential for normal activity. Its deficiency leads to glycogenosis type Iasp [7].

The microsomal glucose-6-phosphate transport protein (T1; translocase) was recognized through the study of glycogenosis type Ib. T1 catalyzes the transport of glucose-6-phosphate into the lumens of hepatic microsomes [76, 77]. In its absence, the liver is unable to release glucose from glucose-6-phosphate. The glucose-6-phosphatase catalytic protein is normal and can be assayed if membranous elements of the liver cell are disrupted by freezing or treatment with detergents, but *in situ* the system is nonfunctional [3, 78, 79]. The defect is also demonstrable in leukocytes, which have impaired uptake of glucose [80] in type Ib, and this may provide a way to test for the disorder. Activity against pyrophosphate and carbamylphosphate is not impaired.

The glucose-6-phosphatase system also depends on the transport of glucose. A number of glucose transport proteins has been identified and they have been designated GLUT 1–6. Deficiency of GLUT 2 causes the syndrome of hepatic glycogenosis in the Fanconi-Bickel syndrome [81].

In glycogenosis Ia, immunochemical assay has indicated an absence of glucose-6-phosphatase catalytic enzyme protein in some patients in whom there is little or no activity, but most have had a normal amount of a protein of normal size [82]. Among patients with partial deficiencies, some have reduced levels of immunoprotein and others normal amounts [14, 82, 83]. In some, the Km is elevated.

The gene for human hepatic glucose-6-phosophatase on chromosome 17q21 has been cloned, and a number of mutations have been identified [84]. These include an arginine-to-cysteine change at amino acid 83 (p.R83C) and p.Q347X a change from a glutamine to a stop codon, which are common in Caucasians. p.R83C is also common in Chinese patients [12]. The most prevalent mutation in Japanese was p.G727T [85]; the next most prevalent was p.R170X.

The cDNA for the translocase deficient in Ib has been cloned [10] and localized to chromosome 11q23 [86]. Common gene mutations in Caucasian patients are G339C and 1211delCT [87]. W118R is common in Japanese [88]. Of the two Japanese patients with type Ib without neutropenia, one had R415X, which had previously been encountered in patients with neutropenia, on one allele and G339D on the other. The other patient was homozygous for G794A, which led to a splicing error, deleting exon 3. Another patient with neutropenia, with abnormal neutrophil function and recurrent infections typical of Ib, was found to have no mutations in the translocase, but to be homozygous for G188R in the glucose-6-phosphatase gene; so, she had type Ia [89]. In a study of adenomas in glycogenosis Ia, alterations in chromosome 6, such as a gain of 6p and a loss of 6q were prominent [90]. Tumors with changes in chromosome 6 tended to be larger than those without.

TREATMENT

Since patients with glycogenosis type Ia are at risk of death or hypoglycemic damage to the brain in early infancy, prompt diagnosis, the avoidance of fasting and the provision of free-glucose are important in getting the patient through this critical period. Infections are particularly dangerous, and the patient may require admission to hospital and treatment with parenteral fluids containing glucose and electrolytes. There is a distinct tendency for improvement with age, even by the age of four or five years [17, 30]. By the time of puberty, considerable amelioration has often been observed [12]; the enlarged liver takes up considerably less of the abdomen and hypoglycemic symptoms are much less prominent. However, little improvement in long-term prognosis as a result of treatment occurred until recently. Portacaval shunting has essentially been abandoned in the treatment of this disease, but it was noted that the parenteral alimentation attendant on the procedure led to reduction in hepatic size and reversal of metabolic abnormalities, the growth failure and the bleeding diathesis [91, 92]. These observations focused attention on approaches to the more regular provision of glucose to meet tissue needs. The approaches that have been successful are continuous nocturnal nasogastric or gastrostomy feeding [92–95] and oral uncooked cornstarch [95, 96].

With either regimen, frequent high carbohydrate meals in which 65–70 percent of the calories are carbohydrate are employed during the day. Dietary intake of fructose and galactose is restricted in some centers and not in others.

Uncooked cornstarch provides glucose in a slow-release fashion. Use in an infant is recommended at a

dose of 1.6 g/kg every four hours. In older children, the requirement is 1.75–2.5 g/kg every six hours, prepared in a 1:2 weight:volume mixture with water or diet drinks [97–100]. Argo (PC International) is prepared by suspension at room temperature. The optimal amount for each patient is determined individually; satisfactory results have been confirmed with 1 g/kg every six hours. Older patients may not require a feeding in the middle of the night if larger quantities (2–4 g/kg) can be taken at bedtime. This regimen has been shown to maintain euglycemia and to reverse clinical and biochemical disturbances in most patients [97, 98]. The age at which cornstarch may be introduced is controversial. Some start at six months and some at 12 months, employing maltodextrin prior to that. We have tended to begin with Polycose®.

Nocturnal nasogastric feeding has also been introduced in infancy at diagnosis. Glucose, Polycose, and elemental formulations have been employed, each providing 8–10 mg/kg per minute in an infant and 5–7 mg/kg per minute in an older child. Clinical and biochemical abnormalities can be reversed and hypoglycemia avoided. Liver size regresses, and the bleeding tendency is reversed [101]. There is a tendency toward the development of hypoglycemia in the morning after the nocturnal feeding is stopped; so that the first meal should be within 15–30 minutes of discontinuing the nocturnal feeding. Hypoglycemia and death have been reported following malfunction of the pump or dislodging the tube [101]. Some patients have required a combination of cornstarch and nocturnal nasogastric feedings. Patients with glycogenosis type Iasp and Ib should also be managed with these regimens. In type Ib, granulocyte and granulocyte–macrophage colony-stimulating factors have been employed to combat the neutropenia and treat the inflammatory disease [102].

Both regimens have been employed long enough to have provided [103] encouraging long-term effects on the course of the disease [97, 98, 103, 104]. Growth has been rewarding, and it is clear that normal adult height may be reached. Some children have remained short [103]. Surprisingly, treatment has been reported to be associated with an absence of development of hepatic adenomas [100] and regression of adenomas has been observed. Proximal renal tubular function has improved [98]. Whether treatment will prevent glomerular dysfunction and renal failure is not clear.

Guidelines for the management of glycogenosis type I are based on consensus of the European study [105]. Overall recommendations are for 60–65 percent of total calories from carbohydrates and 10–15 percent from fat. ACMG standardized Guidelines [12] recommended 60 to 70 percent of calories from carbohydrates and 10 to 15 percent from protein with <30 percent for children older than two years of age.

Shah and O'Dell [106] have recently found uncooked cornstarch is more effective in preventing nocturnal hypoglycemia than continuous nocturnal gastric drip-feeding. Arguments against this conclusion have been published [107]. In our view, the risk of catastrophic hypoglycemia with malfunction of tube or pump is an argument in favor of cornstarch.

Allopurinol is used to lower the concentration of urate to normal levels. A starting dose of 10 mg/kg was recommended. In patients requiring surgery, the bleeding time should be determined. If it is prolonged, it may be improved by 24–48 hours of IV glucose or L-deamino-8-D-arginine vasopressin (DDAVP) [108]. Angiotensin converting enzyme inhibitors have been recommended [105] for hypertension. Hypertriglyceridemia has been treated with nicotinic acid or fibrates [105].

Renal transplantation performed because of renal failure did not improve glucose homeostasis in this disease [109]. Transplantation of the liver provides a definitive cure of the disease [110, 111]; however, the magnitude of the procedure has tended to make its use reserved for a small number of patients with refractory disease or, of course, hepatic malignancy. Caution continues to be the consensus opinion [12].

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60

Glycogenosis type II/Pompe/lysosomal α-glucosidase deficiency

MAJOR PHENOTYPIC EXPRESSION

Cardiomegaly and congestive cardiac failure, macroglossia, weakness of skeletal muscles, accumulation of glycogen in lysosomes of cardiac and skeletal muscle, and absence of acid α-1,4-glucosidase.

INTRODUCTION

Glycogenosis type II has been referred to as generalized glycogenosis because the defect is present in all cells. Clinical expression is most prominently manifested in the heart and, therefore, the disease has been considered as cardiac glycogenosis. It was first described by Pompe [1] in an infant who had died of what had been called idiopathic cardiac hypertrophy. The sections stained with Best carmine identified the material as glycogen. This material was then found in a variety of other organs. Pompe recognized the possibility that a number of other patients diagnosed as having idiopathic cardiac hypertrophy might represent examples of glycogenosis, and he called attention to the report of vacuolization in the myocardium of such a patient previously reported by Sprague and his co-workers [2]. The tissue from this patient was then re-examined and found to contain glycogen [3]. In this family, a number of patients had died of cardiac disease in early infancy. A major impact on the understanding of this disease was made by Hers [4], who identified in lysosomes an α-glucosidase that was active at acid pH and cleaved glycogen, as well as maltose (Figure 60.1). He further documented that this enzyme was deficient in tissues of patients with Pompe disease.

Pronunciation has become interesting in this disease. Many in the United States have pronounced it as if it were spelled pompy, while others more pedantic, such as the *New York Times* [5], have corrected us indicating that it should be pronounced like the city in Italy that was buried by the volcano. Actually, Pompe was an Austrian; the *Oxford English Dictionary* indicates the e should be pronounced like 'her' or 'bird'.

With the discovery of the enzyme defect, it soon became apparent that there were adult-onset forms of the disease. In some patients, this is manifest only in exercise avoidance; others are wheelchair-bound and/or develop diaphragmatic failure.

These discoveries launched the field of lysosomal storage diseases. The gene for the enzyme has been localized to chromosome 17q25 [6]. It is now recognized that there

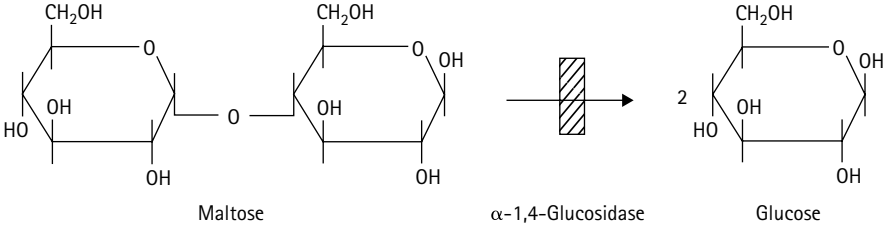

Figure 60.1 α-1,4-Glucosidase, the site of the defect in Pompe disease. This enzyme catalyzes the cleavage of other linear oligosaccharides, as well as of maltose.

are purely myopathic late onset forms of α-glucosidase deficiency and a spectrum of clinical phenotypes between that and the classic infantile Pompe disease [7]. Enzyme activity gernally correlates with the degree of clinical severity. The gene has been cloned and a number of mutations has been identified [8]. A few have been identified with various ethnic groups [9, 10]. Among three mutations common in Dutch adult-onset myopathic patients 63 percent of patients carried one or two of them, (IVS 1-13T-G, 525delT and EX18DE) [11]. Enzyme replacement was first shown to be effective in quail [12].

CLINICAL ABNORMALITIES

The classic infantile form of glycogenosis II (Figures 60.2–60.9) is of rapidly progressive cardiomyopathy with massive cardiomegaly and death within the first year [13]. The discovery of the enzyme defect led to the recognition of a spectrum of variants. The clinical picture of those at the other end of the spectrum is that of adult-onset skeletal myopathy. Many variants have been observed between these two extremes.

In the infantile disease, manifestations begin in the first weeks or months of life [13, 14]. Symptoms may even be noted at birth. Poor feeding and failure to thrive may be early complaints, but cyanosis and attacks of dyspnea begin promptly, and there is rapid progression to intractable cardiac failure [15]. Death is by congestive failure, sometimes with a complicating terminal pneumonia.

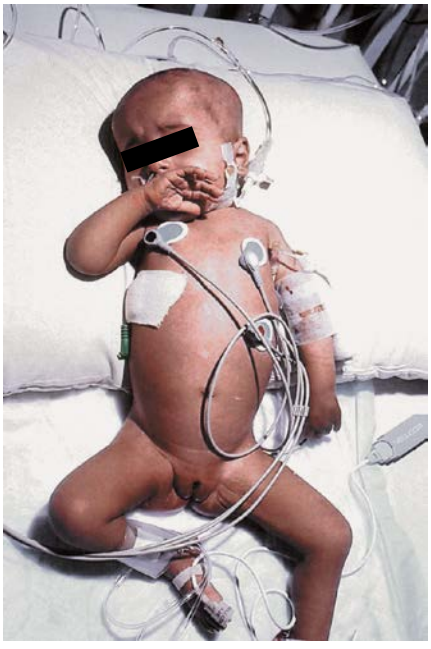

Figure 60.2 A six-month-old infant with glycogenosis type II. She illustrates the severe hypotonia with which she presented. She also had cardiomyopathy and died at nine months of age of pneumonia.

Physical examination reveals signs of cardiac failure and the hallmark feature, cardiomegaly. Massive enlargement of the heart is visible on roentgenograms (Figure 60.8). Significant cardiac murmurs are not usually present [16].

Hepatomegaly may be seen once cardiac failure begins, but it does not result from massive storage of glycogen in the

Figure 60.3 AMS: An eight-month-old boy with Pompe disease. He was flaccid and intubated and had dilated cardiomyopathy.

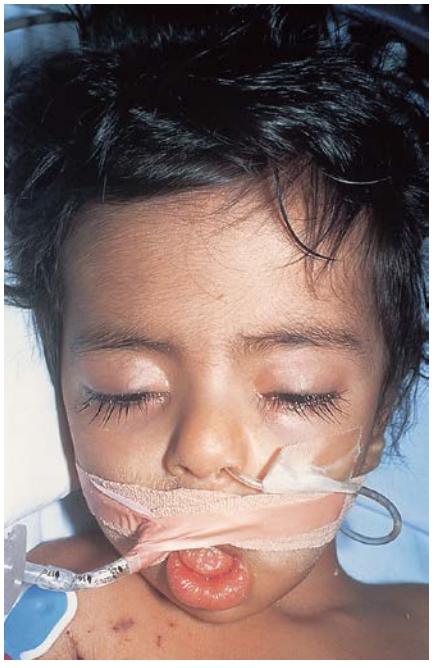

Figure 60.4 AMS: The lips were thick and the tongue appeared large. The level of activity of α-glucosidase in fibroblasts was 3 percent of control. The V_{max} in muscle was markedly reduced.

Figure 60.5 AAAD: A six-month-old infant with Pompe disease. Dyspnea and cyanosis began at two months. A sibling died at four months. The liver was palpable 4 cm below the costal margin. There was a grade I–III systolic cardiac murmur. Electrocardiogram (EKG) and echocardiogram revealed biventricular enlargement and poor left ventricular contraction.

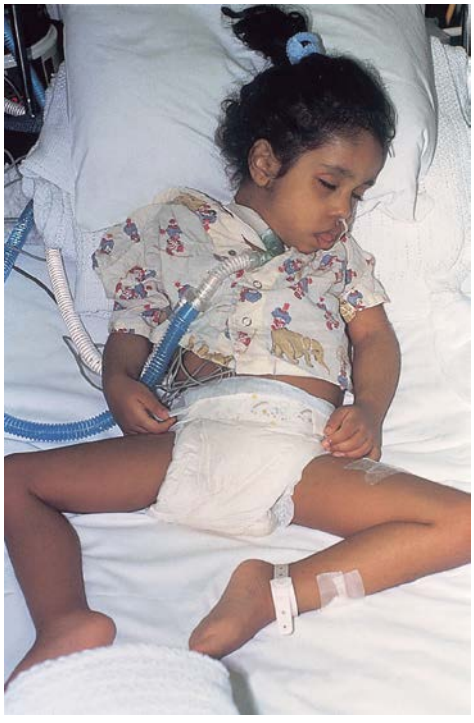

Figure 60.6 A girl with Pompe disease. The position was flaccid. She had a tracheostomy and required nasogastric feeding.

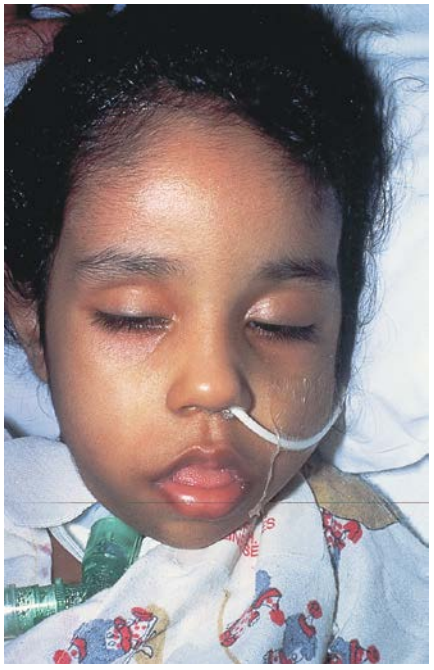

Figure 60.7 The lips were full and the tongue quite large.

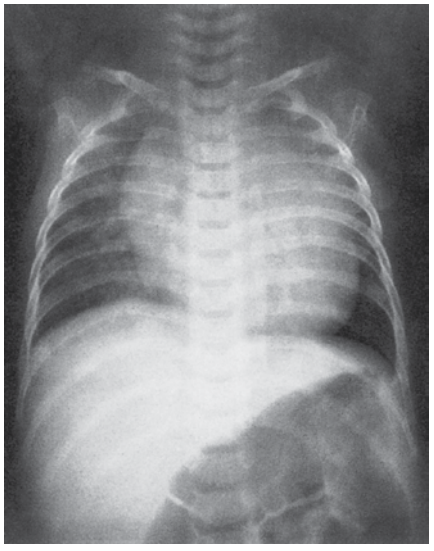

Figure 60.8 AK: A four-month-old infant with Pompe disease, had evidence of cardiomegaly on Roentgenographic examination. (Courtesy of Dr MS Ahmad, Dharan Health Centre, Dharan, Saudi Arabia.)

liver. The electrocardiogram (Figure 60.9) may show very large QRS complexes in all leads and a short PR interval [17]. Left-axis deviation or an absence of the normal right-axis deviation of this age and evidence of biventricular hypertrophy are seen, as well as T-wave changes and depression of the ST segment. Cardiac catheterization or echocardiogram shows biventricular hypertrophy and obstruction of left ventricular outflow [18]. Death within the first year of life is the usual course in this disease, but patients have been reported [19] in whom rapidly

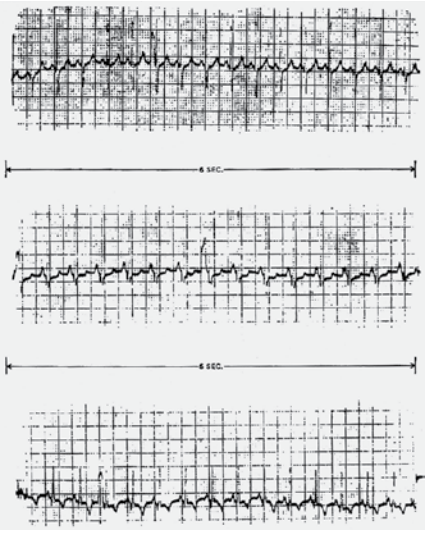

Figure 60.9 AL: The electrocardiogram (EKG) revealed biventricular hypertrophy. The leads shown, from the top, were 1, 2, and 3. (Courtesy of Dr MS Ahmad, Dharan Health Centre, Dharan, Saudi Arabia.)

progressive cardiomegaly and cardiac failure led to death shortly after onset as late as 11 and 15 years.

Classification on the basis of age of onset appears less meaningful now that genetic variation can be expressed in terms of enzyme activity and the nature of mutation. In fact, atypical or nonclassic forms of the disease have been reported with onset in infancy [20]. Most of these infants had left ventricular hypertrophy, but they did not have left ventricular outflow obstruction. Death occurred later than the first year, often from myopathy-related pulmonary disease.

Skeletal muscle disease is prominent in all infantile patients. It is manifested by marked hypotonia and weakness associated with diminished or absent deep tendon reflexes. The clinical picture may be suggestive of amyotonia congenita [21]. Muscle mass is normal, but the muscles may feel hard. Classically, the tongue is enlarged. A protuberant tongue (Figures 60.7 and 60.10) with failure to thrive, hypotonia, a protuberant abdomen, and possibly an umbilical hernia may suggest a diagnosis of hypothyroidism or Down syndrome. Macroglossia is caused by infiltration of the muscle fibers of the tongue with glycogen, but macroglossia is noted in fewer than half of patients. Among 12 adult-onset patients 11 had ptosis [22]; it was the presenting feature in three.

A small number of patients present in infancy or early childhood with a predominantly skeletal muscle disease without cardiac disease [23, 24]. They may have lordosis or scoliosis, and may require surgical treatment. There may be localized pseudohypertrophy [25]. These patients display a more slowly progressive disease and death occurs by 19 years from pneumonia or respiratory failure. An emerging phenotype has been observed in patients with infantile Pompe disease, surviving "long term" (≥5 years) [26]. All had low anti-glucosidase antibodies. All had improved cardiac features, and 11 were independently ambulatory.

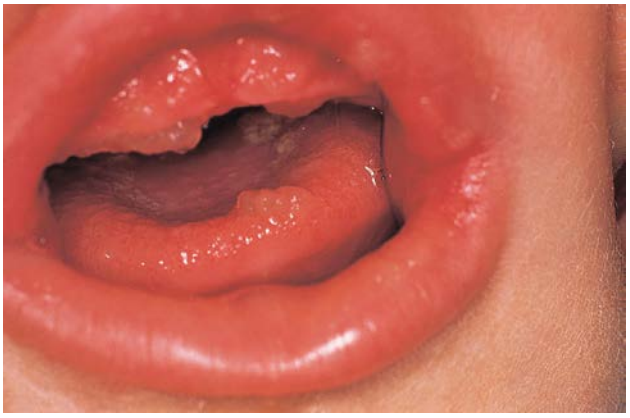

Figure 60.10 AAAD: The tongue appeared large and the lips thick. Biopsy of the right gastrocnemius revealed distention of the myocytes with stored glycogen. Activity of α-glucosidase was very low.

In a report [27] of 20 infantile patients from Three Netherlands and 133 from the literature who had similar courses, median age at death was 7.7 and six months respectively; and only 5 percent and 8 percent survival beyond one year.

A distinct group of patients has presented in the second or third decade with muscular weakness. This is the group that has been referred to as having adult-onset disease (Figure 60.11). The clinical picture in these patients is that of a slowly progressive myopathy with little or no cardiac abnormality [23, 28, 29]. One patient presented with unexplained hair loss [5]. In 12 patients with adult-onset disease, four had ptosis [30]. This may be an alerting signal of the disorder. These patients may also

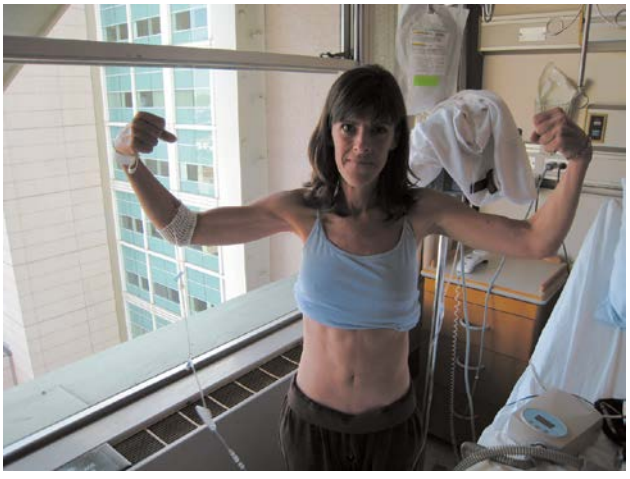

Figure 60.11 AB: A 45-year-old woman with Pompe disease. Her work that involved packing and lifting boxes led to impressive arm muscles, but she was already experiencing diaphragmatic weakness; she required assisted ventilation at least at night.

die of respiratory failure [26]. A patient with this form of the disease was reported to develop a Wolff-Parkinson-White syndrome and a secondary atrioventricular block [31]. The early phenotype is that of a progressive proximal muscle weakness [32]. The legs and paraspinal muscles are particularly involved. Some patients present with back pain. Deep tendon reflexes may disappear. Urinary incontinence may indicate myopathy [33]. Diaphragmatic involvement may present as sleep apnea [34], but ultimately leads to respiratory failure or pneumonia.

Electromyography (EMG) in any of the forms of type II glycogenosis reveals pseudomyotonia and high-frequency discharges and fibrillations [35]. Creatine kinase (CK) is usually elevated. It may be up to ten-fold in infantile patients. It may be normal, but its elevation can serve as the alerting signal for the diagnosis in a myopathic adult [36]. Transaminases may also be elevated [37]. Type II glycogenosis differs from the other glycogenoses in that no other abnormalities are detectable in the clinical chemistry laboratory. There is no hypoglycemia, and concentrations of lactic acid, uric acid, and lipids are normal.

The histopathology of this disease is one of generalized deposition of increased amounts of glycogen throughout the body, but without the enormous increase in storage that tends to increase the size of the liver massively in type I and type III. The material usually stains basophilic in hematoxylin and eosin, and red with special stains for glycogen (Figure 60.12). It is digestible with amylase and contains phosphates [38]. The typical lacework appearance of sections of the myocardium results from the deposition of glycogen in cardiac fibrils. In the electron microscopic picture (Figure 60.13), it is clear that the glycogen is membrane-bound, and that the accumulation is within the lysosome in contradistinction to the appearance of other glycogenoses [39]. However, in the muscle and in the heart

Figure 60.12 Periodic acid Schiff (PAS)-stained longitudinal section of muscle of a patient with Pompe disease. The infant presented as very floppy and had a severe deficiency of acid maltase. The red-staining accumulations of glycogen were dramatically visible. (Courtesy of Dr John S Romine, Department of Neurosciences, University of California, San Diego, La Jolla, California.)

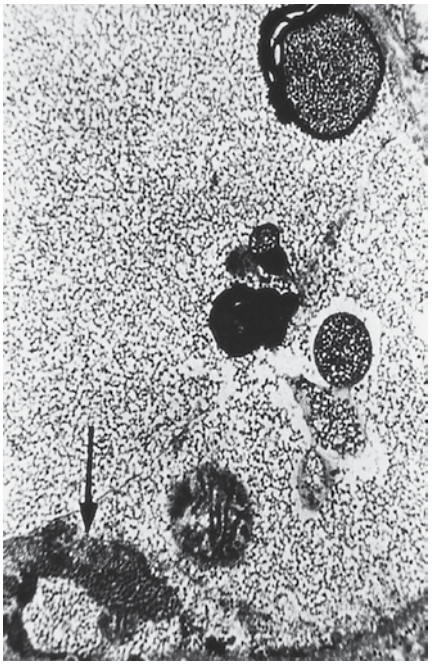

Figure 60.13 Electron micrograph of the muscle of the same baby demonstrates the extensive accumulation of glycogen. Most of the muscle fiber was replaced by glycogen granules. The arrow in the slide points to a remnant of a muscle fiber. Also evident are membrane-bound collections of glycogen. (Courtesy of Dr John S Romine, Department of Neurosciences, University of California, San Diego, La Jolla, California.)

of type II patients, there is also cytoplasmic accumulation of glycogen, and this has been correlated with destruction of contractile elements.

In addition to the accumulation in muscle and liver, deposition may be seen in motor nuclei in the brainstem and in the anterior horn cells of the spinal cord [22]. There is only slight deposition in the neurons of the cerebral cortex, but it may even be seen in fetal life [40]. Histologic examination of the adult-onset patient, in whom the heart is clinically normal, may reveal no accumulation of glycogen in cardiac muscle, even though the enzyme defect is no different there than in skeletal muscle [41]. The histologic appearance of skeletal muscle of infantile and adult-onset patients is indistinguishable [41]. Glycogen deposition may not be seen in the heart, liver, or brain of patients who have an adult onset. In classic infantile patients, the diagnosis may also be made by electron microscopic examination of biopsied skin [42], in which characteristic accumulation of glycogen within a lining membrane may be demonstrated.

GENETICS AND PATHOGENESIS

Type II glycogenosis is inherited in an autosomal recessive fashion. Consanguinity has been observed. Prevalence is high in Taiwan and South China; estimated frequency was 1 in 50,000 [43]. Many patients are seen in Saudi Arabia (see Figures 60.2, 60.3, 60.4, 60.5, 60.6, 60.7, 60.8, 60.9,

and 60.10). Elsewhere, the disease is rare. The molecular defect in type II glycogenosis is in α-1,4-glucosidase (see Figure 60.1) [4]. The activity of this lysosomal enzyme in human liver at pH 4.0 is between three and ten times that at pH 7.4 [44]. It is active against maltose and other oligosaccharides, as well as glycogen. The product is free-glucose [45]. The enzyme is normally widely distributed in tissues, and it is present in fibroblasts, leukocytes, and amniocytes. In Pompe disease, deficiency of the enzyme has been demonstrable in all tissues measured [23, 28, 29], and this generalized deficiency is true of patients regardless of clinical phenotype. This explains the intralysosomal accumulation of glycogen in organs such as the liver, where it would be inaccessible to other enzymes of glycogenolysis, such as phosphorylase and the debranching enzyme. In organs containing these enzymes, glycogen is not found outside the lysosomal fraction [46].

The diagnosis is best made by assay of the enzyme in muscle or fibroblasts [47]. The enzyme can be measured in transformed lymphoblasts or lymphocytes, but it may not be reliable in unfractionated leukocytes unless antibody against the enzyme is employed to prove that the activity being measured is not an unrelated glucosidase [48, 49]. Among groups of patients with different clinical phenotypes, there was an inverse correlation between the severity of clinical manifestation and the level of residual enzyme activity [50]. In general, the classic infantile patients display virtually absent activity, while adult-onset patients usually have considerable residual activity, but there is appreciable overlap among patients in between. In the infantile presentation, a catalytically inactive, immunochemically reactive enzyme has been observed. In some patients with the adult phenotype, a reduction in the amount of enzyme protein has been reported [50–52]. Genetic heterogeneity is evident within both the infantile and adult forms of the disease; of nine patients with the infantile form, eight were cross-reacting material (CRM) negative and one was CRM positive [52]. In the adult phenotype, CRM-negative as well as CRM-positive variants have been observed. The enzyme undergoes extensive post-translational modification: seven N-linked glycosylations and phosphorylation of mannose moieties yield the mannose-6-phosphate lysosomal recognition marker.

All of the variants of glycogenosis type II described are inherited in an autosomal recessive fashion. Analysis of affected infants in reported kindreds after subtraction of the probands yielded a figure of 21.4 percent, approximating the 25 percent expected [53]. The incidence of consanguinity was high. Reduced activity in heterozygotes of α-1,4-glucosidase has been demonstrated by assessment of fibroblasts [54]. Heterozygote detection using leukocytes from peripheral blood has been accomplished, but it was unreliable [4]. An assay for heterozygosity was developed for lymphocytes stimulated by phytohemagglutinin [55]. Prenatal diagnosis has been carried out using cultivated amniocytes [56, 57]. Rapid prenatal diagnosis has been accomplished by electron microscopic examination of uncultured amniotic cells [58]. Of 26 fetuses at risk, six were found to be positive. Each of these prenatal diagnoses was confirmed by enzyme assay of amniocytes, and in tissues of three fetuses terminated and in three affected infants delivered. Prenatal diagnosis has also been reported by assay of the enzyme in uncultured chorionic villus material [59].

The nature of the disease is usually quite similar in all affected members of a family. However, families have been reported in which there were examples of the typical infantile Pompe form of the disease and the late-onset adult [60, 61]. This situation has been shown to reflect allelic diversity [62] in which an affected grandparent with adult-onset disease had two mutant alleles, one specifying partial deficiency and one complete. This second allele was passed to a son whose spouse also turned out to have such a gene, and an infantile classic patient was produced [61]. Somatic cell hybridization studies have failed to show evidence of complementation, and so a single locus appears to exist.

The gene has been localized to chromosome 17q25 [6]. The gene has been cloned and the sequence of the cDNA determined [63]. It contains 20 exons and is approximately 20 kb. Mutations have been defined in patients with different phenotypes. Among infantile patients, a majority of those studied have had undetectable mRNA [8, 64, 65]. A number of gross alterations in the gene have been found, such as deletion of exon 18 and stop codons [66, 67]. In contrast, a number of those with adult-onset phenotypes have had missense mutations [68]. Many of the mutations reported have been genetic compounds in which different mutations were on each allele. The number and variety of mutations observed indicate that the degree of heterogeneity in this population will be very great. Expression of a G-to-A transition in exon 11 *in vitro* indicated that the mutation coded for absence of catalytic activity [69]. This is consistent with the infantile onset phenotype. The common mutation in Chinese patients [10] is an Asp645Glu. The African mutation is an Arg854X [9]. Two deletions, del525T and del exon 18, are very common in Holland and in other Caucasian populations [70]. In 40 Italian patients with late onset disease, there were 26 different mutations, 12 novel [71]. The most common was IVS1A-AS-13T>G; it occurred on one allele in 34 patients. Among 98 Caucasian patients who were compound heterozygotes for this -13T>G mutation, there were a variety of second deleterious mutations, glucosidase activity was 3–20 percent of control, and there was a wide spectrum of clinical phenotypes [72]. Twelve different C.-13T>G haplotypes were observed. All of 22 Spanish patients with the c.-13T>G mutation were of the same haplotype, consistent with a founder effect [73]. Two novel mutations, P361L and R437 were found in a 16-year-old Chinese patient [74]. His 13-year-old asymptomatic brother had the same mutation. In 40 Italian patients with late-onset disease, 12 novel alleles were found [75]. The common c.-32-13T>G was found in 85 percent of patients.

The molecular biology of this gene is complicated by the fact that considerable polymorphism has been identified in individuals with no disease or enzymatic abnormality. Eleven restriction fragment length polymorphisms (RFLPs) have been identified which result from substitution of bases within introns [64], but silent mutations in the coding regions have also been observed [63]. These RFLPs may be useful for heterozygote detection and prenatal diagnosis. Prenatal diagnosis has been carried out in a family in which the mother carried a ΔT525 deletion, and whose previous child had died of glycogenosis II [76]. Mutational analysis correctly identified the absence of the mother's deletion in chorionic villus material, and enzyme analysis in the fetus was normal. If the mutation is known, this is a convenient method of prenatal diagnosis [77].

Newborn screening has been conducted in Taiwan since 2005 [78]. Activity of α-glucosidase was measured in spots of dried blood. In 13 patients, later onset Pompe disease was diagnosed and mutations were found. A confounding issue in diagnosis is the occurrence of patients with cardiomyopathy and lysosomal storage of glycogen in whom the activity of acid α-glucosidase is normal [78, 79]. Arrhythmias, especially Wolff-Parkinson-White syndrome, were common in these patients. Most of those patients had impaired mental development.

TREATMENT

Supportive therapy, including ventilator assistance, is useful especially in advanced myopathic disease. Bone marrow transplantation was accomplished in cattle with α-glucosidase deficiency, but there was no effect on the disease [80].

Enzyme replacement therapy was employed early, using acid maltase purified from Aspergillus and human placenta, without clinical evidence of improvement. Recognition of the importance of the mannose-6-phosphate receptor-mediated lysosomal uptake of enzymes and the development of recombinant human enzyme have completely changed this area of investigation. IV administration in acid glucosidase-deficient quail led to increased enzyme activity in muscle and a decrease in glycogen content to normal. Clinically, the birds righted themselves and even flew [12]. A clinical trial of recombinant human enzyme produced in rabbit milk was published [81], including evidence of improvement in muscle histopathology, decrease in cardiac size and improvement in function.

The modern era of treatment was ushered in by the infusion of recombinant human α-glucosidase in three infants with infantile disease. There was a decrease in cardiac size and maintenance of normal function for more than one year at report [82]. Enzyme replacement therapy has been reported to be effective, especially in infants [83]. Enzyme replacement therapy was summarized in 40 patients with infantile-onset disease diagnosed between 1983 and 2008 in Taiwan [84]. There were five presymptomatic patients diagnosed by newborn screening. Regression of cardiomyopathy, lowering of B-type natriuretic peptide, and improved survival were recorded following treatment. Nevertheless, two died and two required ventilator support. Despite improvement in the electrocardiogram (EKG), three had life-threatening arrhythmias. None were in the newborn screening group. In the late-onset Pompe patients diagnosed by newborn screening [78], enzyme replacement therapy was initiated when symptoms or elevation of CK occurred. In a study of treatment in late-onset Pompe disease, 90 patients were randomized 2:1 to enzyme replacement or placebo [85]. Cardiovascular abnormalities were identified, such as elevated left ventricular mass, a short PR interval, or decreased left ventricular function in 5–10 percent. There was no change in cardiac status in response to enzyme replacement therapy. Among 90 patients randomized to enzyme replacement or placebo [86], there was no improvement in cardiac status. In assessment of the metabolic myopathy the six-minute walk test was used as a marker [87]. After 78 weeks of enzyme or placebo, there was significant improvement in walking distance, and stabilization of pulmonary function as measured by forced vital capacity (FVC). Mean increase in distance walked was 25 percent in the treatment group and 3% in the control. In mouse models, accumulation of glycogen in cervical spinal cord was great, indicating that therapy targeted to muscle maybe ineffective [88].

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61

Glycogenosis type III/amylo-1, 6-glucosidase (debrancher) deficiency

MAJOR PHENOTYPIC EXPRESSION

Hepatomegaly, hypoglycemia, late myopathy, storage of glycogen in liver and muscle, elevated transaminases and creatine phosphokinase, and deficient activity of the glycogen debranching enzyme amylo-1,6-glucosidase.

INTRODUCTION

The first patient described with glycogenosis type III was reported by Forbes in 1953 [1]. She had been noted at one year of age to have a large abdomen, and when she presented at 3.4 years, the liver was palpated at the left iliac crest. By 13 years, she was described as not appearing chronically ill [2]. The liver was studied by Illingworth and the Coris [3, 4], who found the glycogen content of both muscle and liver to be increased. The structure of the glycogen was very abnormal and resembled a phosphorylase limit dextrin (Figure 61.1). The outer chains were abnormally short and the number of branch points was increased. The structure of the glycogen suggested that the defect was in the debrancher enzyme. This hypothesis was promptly confirmed by assay of the enzyme [4]. Activity of amylo-1,6-glucosidase was virtually absent in liver and muscle [4, 5]. The history of the disease is impressive in that the nature of the disorder and the enzyme defect were worked out in studies on the index patient within a few years of the first report. The enzyme has two independent catalytic activities, glucosidase (EC 3.2.1.33) and transferase (EC 2.4.1.25). The cDNA has been cloned, and heterogeneity has been demonstrated in study of mRNAs [6]. The gene has been mapped to chromosome 1p21 [7]. A number of mutations has been described in a considerable variety of patients [8].

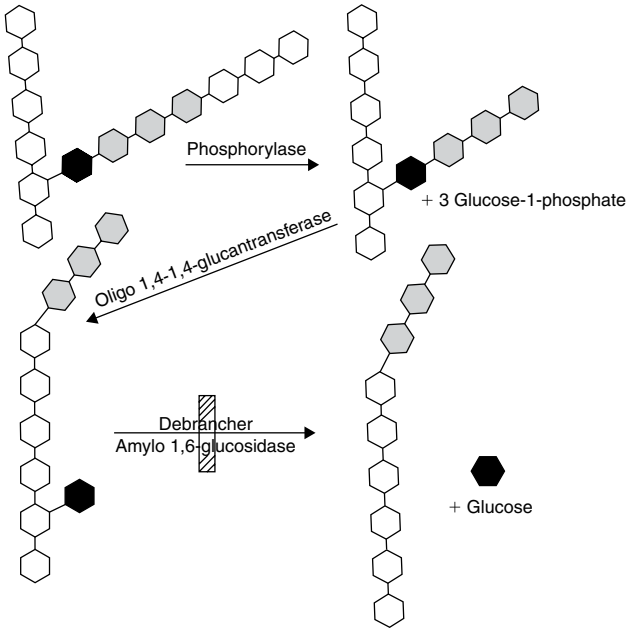

Figure 61.1 The sequential debranching of glycogen. Phosphorylase catalyzes the cleavage of glycosyl units in α-1,4 glucose unit before the amylo-1,6-glucosidase can cleave the glucose moiety in 1,6 linkage. This debrancher is the site of the defect in glycogenosis type III.

CLINICAL ABNORMALITIES

The clinical manifestations of glycogenosis III tend to be milder than those of type I (Chapter 59), but the diseases cannot reliably be distinguished without laboratory procedures. Death in infancy has been recorded [2]. The most consistent clinical feature is hepatomegaly and it may be the only clinical abnormality at the time of presentation (Figures 61.2–61.7) [1]. In contrast to the patient with type I, these patients may also have some enlargement of the spleen [9]. The kidneys are not enlarged in this disease; in fact, assessment of renal size by imaging of the abdomen may aid in distinguishing types I and III [10]. The enlargement of the liver may be massive; in infancy, it may interfere with walking or even standing. A two-year-old patient of ours simply toppled over if not supported in the standing position.

With time and growth, the patient's size tends to catch up with the liver which, while prominent, is less impressive.

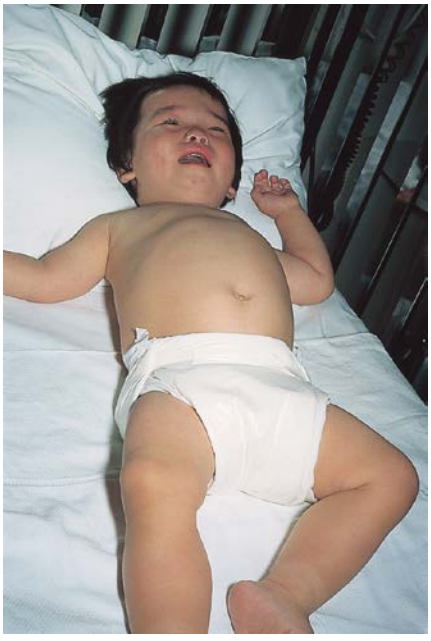

Figure 61.2 JL: An 18-month-old boy with glycogenosis type III [12] who presented with a history of increasing abdominal protuberance. He woke routinely at 4 a.m. for breakfast. (Illustration kindly provided by Dr Jon Wolff.)

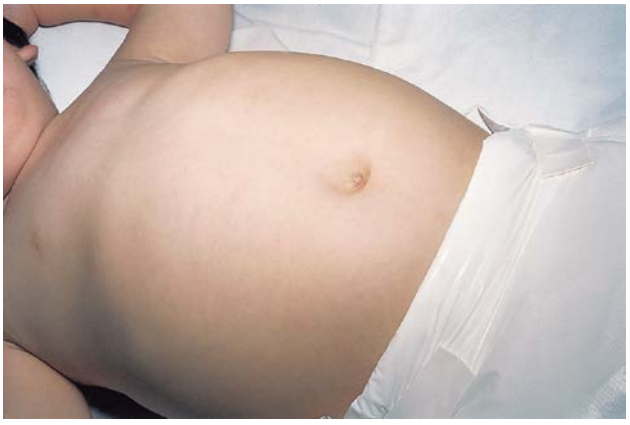

Figure 61.3 JL: Close-up of the abdomen highlighted the relatively enormous size of the liver. With time, he grew and the liver could only be found by palpation. (Illustration kindly provided by Dr Jon Wolff.)

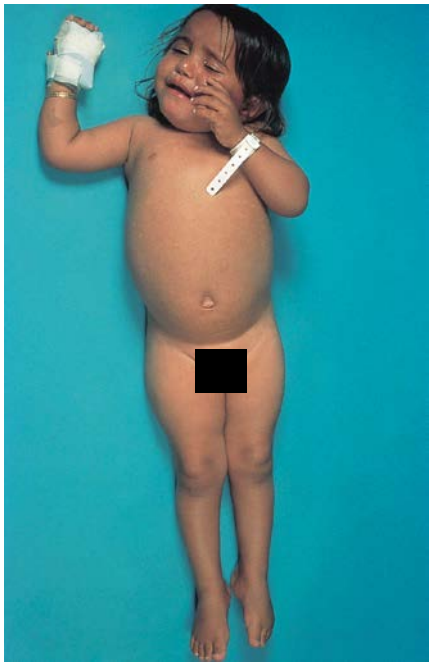

Figure 61.4 SH: An infant with glycogenosis type III with protruberant abdomen.

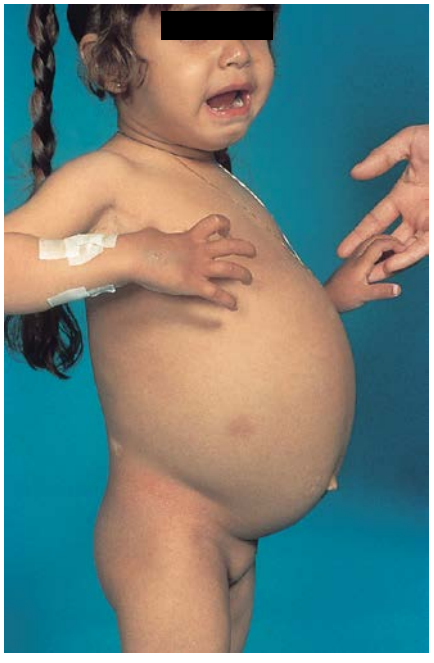

Figure 61.5 MS: This infant with glycogenosis type III had a highly prominent abdomen.

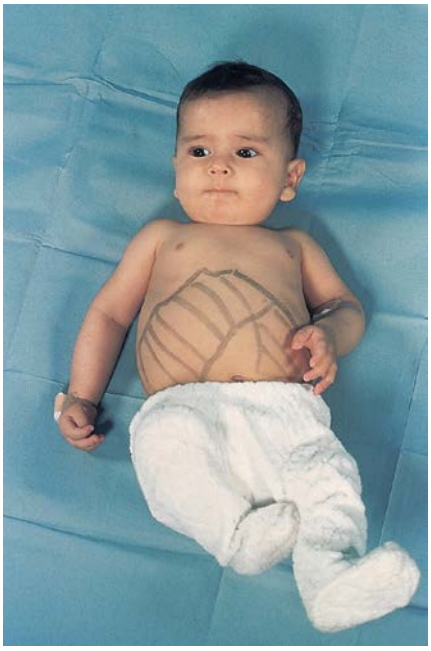

Figure 61.6 FMS: The enlarged liver created a very protruberant abdomen of this infant with glycogenosis type III.

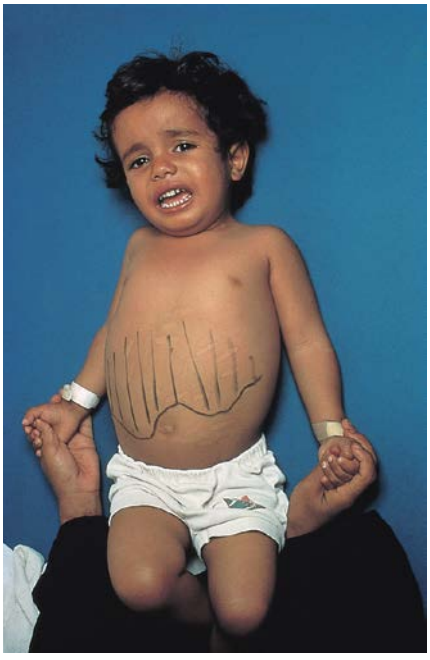

Figure 61.7 Another girl with glycogenosis type III and marked hepatomegaly.

By adulthood, the abdomen usually appears normal [2]. The size of the liver may be normal by puberty [11].

Hypoglycemia is not usually a prominent feature of this disease, but fasting concentrations of glucose are usually moderately reduced, and some patients, especially in infancy, had severe hypoglycemia (Figures 61.8 and 61.9) and even convulsions. Some patients have developed mental impairment [12], presumably as a consequence of

Figure 61.8 A five-year-old girl with glycogenosis type III. Unlike most patients with this disease, she had problems with hypoglycemia and was using an overnight glucose drip. Introduction of a cornstarch regimen at this time permitted its discontinuation.

Figure 61.9 Three siblings with glycogenosis III who presented with hypoglycemia and elevated transaminases. Only the protuberant abdomen of the youngest suggests abnormality.

hypoglycemia. It is thought that hypoglycemia of infancy and early childhood may reflect developmental inadequacy of gluconeogenesis [10], but the behavior may also modulate the problem. For instance, one of our patients ate five meals a day and woke at 4 a.m. for breakfast [13]. In any case,

by the second decade fasting hypoglycemia improves, and most adults with this disease tolerate fasting well [10]. Ketonuria may be observed after a moderate fast when the blood glucose approximates 40 mg/dL [14]. Hyperketonuria tends not to lead to metabolic acidosis or symptoms. In a study of various glycogenoses, the patients with type III all developed ketotic hypoglycemia [15].

Vigorous catabolism of fatty acids and hyperactive fasting ketonuria are indicated by a ten-fold elevation in concentrations of 3-hydroxybutyric acid after a 12-hour fast. Concentrations of lipids may be elevated, but not to the degree seen in von Gierke disease [1, 16]. The concentration of cholesterol may be elevated in the absence of hypertriglyceridemia and there may be hyperbetalipoproteinemia [10]. Over the years, levels of lipids in the blood tend to decrease [1] and those of sugar to increase. Patients with type III disease do not develop xanthomas. Concentrations of uric acid are normal, and levels of lactate are also usually normal. Concentrations of transaminases are usually elevated in infancy, and this may make the clinical picture confusing, suggesting hepatitis or hepatocellular insult as a cause of the hepatomegaly. On the other hand, the creatine kinase (CK) is usually elevated [13], so some of the enzyme levels could represent myopathy. Nevertheless, the histologic appearance of the liver usually indicates at least some fibrosis in this disease [9, 13]. Age-related change in transaminase activity has been observed, high in infancy and the first decade, followed by progressive reduction to adulthood [17]. Frank cirrhosis may be encountered, but cirrhosis does not usually progress with age [18]. Cirrhosis appeared to be more common in Japan [19] and progressive cirrhosis and hepatic failure have been observed, particularly in that country [19, 20]. Hepatic adenomas have been described [21]. They have not usually developed malignant change, but hepatocellular carcinoma has been reported [22] in a 63-year-old man. Therefore, surveillance is necessary. Hepatocellular carcinoma has been observed in end-stage cirrhosis [18, 20]. Ultrasound has been useful in following the progress of liver disease. Of 44 patients, by the third decade, no one had normal hepatic ultrasound [16]. Only two of these patients developed hepatic failure and required transplantation. Pallor has been described [23], but patients are usually not anemic.

Growth and development may be completely normal in this disease [1], but in some patients, impairment of linear growth may be striking [13]. Many children are of normal size, although in a lower percentile for height than for weight and head circumference.

Renal tubular acidosis has been reported in two patients, along with severe failure to thrive [24]. One of the patients had a typical distal renal tubular acidosis, while the other had glycosuria and bicarbonate wasting, suggesting a Fanconi syndrome, as seen in type I glycogenosis (Chapter 59).

It has been recognized since the first patient, that glycogen accumulates in muscle as well as the liver. It has only more recently been recognized that this may lead to a myopathy, especially by adulthood [25–27]. In fact, the late myopathy is the major morbidity of type III glycogenosis. There may be hypotonia, but muscle atrophy occurs as well as weakness. It is often notable in the interossei and over the thumbs. Atrophy in the legs has suggested diagnosis of Charcot–Marie–Tooth disease [28]. Weakness tends to be slowly progressive. Walking rapidly or upstairs brings out the weakness. Strenuous exercise cannot be effectively performed, but there is no tenderness, cramping, or myoglobinuria. In sustained submaximal exercise testing, patients with this disease could not generally exercise for 60 minutes, a fact readily completed by patients with McArdle disease [25]. Some patients have first presented in adulthood with progressive muscular weakness. Ultimately, the patient may be wheelchair-bound or bedridden. The electromyogram (EMG) may reveal a myopathic pattern with abundant fibrillations [26]. Nerve conduction may be abnormal. Activity of CK in serum is elevated [25] in the presence or absence of myopathic symptoms, but a normal level of CK does not rule out deficiency of debranching enzyme in muscle [29]. Deformity of the chest and kyphoscoliosis may be progressive. Some patients have had muscular fasciculations suggestive of motor neuron disease and peripheral nerve involvement has been documented in this disease [30], despite current reports [31] of 16 adult patients which failed to find abnormal nerve conduction studies [31]. Electron microscopic study of a 20-month-old boy revealed selective massive accumulation of glycogen in the Schwann cells of unmyelinated nerve fibers.

Cardiomyopathy may also occur; in fact, abnormalities of the electrocardiogram and echocardiographic evidence of biventricular hypertrophy are frequently observed, though rarely accompanied by cardiac symptoms [32]. However, congestive cardiac failure has been described [26], as has exertional dyspnea and chest pain, and sudden death may occur [33].

Polycystic ovaries have been described in this disease, as in type I, but without effect on fertility [34].

The mineral density of bone has been reported to be reduced in nine patients with Type III disease [35].

Dysmorphic features, hypoplasia of the midface, depressed nasal bridge, broad up-turned nasal tips, indistinct filtral pillars, and bow-shaped lips have been described [36]. We have not seen this in our patients.

Histologic examination reveals the cells of the liver to be swollen and finely granular with an open nucleus. The material stored may be identified by Best stain as glycogen. Large vacuoles in the hepatocytes may be filled with periodic acid Schiff (PAS)-positive material. There is evidence of an increased amount of fibrous tissue within the lobules of the liver. In addition, there may be some proliferation of the bile ducts. Unlike the picture in glycogenosis type I, there is no infiltration with fat. Histologic examination of the muscle reveals abundant amounts of glycogen visible in subsarcolemmic areas of myofibrils [17]. The glycogen in this disease is more soluble than a normal glycogen and, therefore, it tends to disappear more readily from

Table 61.1 Features by which type III glycogenosis may be distinguished from type I

Feature	Type III	Type I
Hypoglycemia	+	Severe
Bleeding diathesis	0	+
Splenomegaly	±	0
Enlarged kidneys	0	+
Myopathy	+	0
Elevated CK, transaminases	++	0
Fasting ketogenesis	++	+
Lactic acidemia	0	+
Alanine in plasma	Low	High
Hyperuricemia	0	+
Little or no response to glucagons after fast	+	+
Normal postprandial response to glucagon	+	0
Increase in blood glucose after galactose, fructose, or glycerol	+	0

conventional histologic preparations. Cryostat sections may be useful for biopsies.

A variety of functional studies has been employed to document the presence of type III glycogenosis and to distinguish it from type I (Table 61.1). Among the most useful is the administration of glucagon after a 14-hour fast, following which, there is little or no increase in blood glucose; and again 2–3 hours after a meal, following which, there is a blood glucose response [37]; the rise in glucose may be normal, but it is usually reduced, though clearly present. This is consistent with the availability of glucose moieties on the elongated outer branches of glycogen to degradation of phosphorylase, even in the total absence of debranching activity. Also, these patients do not have lactic acidemia and, in particular contrast to patients with type I, the concentration of lactic acid does not increase after glucagon. Also, in contrast to type I, there is a normal level of conversion of galactose, fructose, or glycerol to glucose [38].

The absence of highly elevated concentrations of lactic acid has been cited [39] as a reason why patients with this disease have been observed to have seizures at higher concentrations of glucose than those with type I disease, in whom the brain may be able to substitute lactate for glucose. On the other hand, seizures are not common in glycogenosis type III and concentrations of ketones in blood are elevated in this disease [40]. Mobilization of fat is very active, as is gluconeogenesis.

Metabolism of amino acids in glycogenosis type III is distinctly different from that of normal individuals and of patients with type I disease [41]. The major difference is in the responses of the principal gluconeogenic amino acid, alanine. The concentrations of alanine in plasma were significantly lower in 11 patients than in 27 controls [41]. This would be consistent with an overactive process of gluconeogenesis. In contrast in type I, concentrations of alanine are increased, consistent with defective gluconeogenesis. In type III, there were significantly lower concentrations of a number of other amino acids, notably threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and lysine. This would be consistent with the operation of the alanine–glucose cycle in which branched-chain and other essential amino acids in muscle are depleted in order to serve as donors of nitrogen for the net synthesis of alanine from pyruvate in muscle [42]. Alanine is the only amino acid whose concentration in venous blood draining muscle is higher than arterial. The alanine formed is then transported through the blood to the liver, where it is converted to glucose.

Following the ingestion of glucose in a glucose tolerance test, concentrations of alanine in the blood rise dramatically in type III patients, while in type I patients they fall [41]. In controls, the level of alanine does not change after glucose. After a protein challenge with 4 g beef/kg, levels of alanine rose, but much less than in normals or than in type I patients and significantly less than the rise in alanine in type III after glucose. All of these data are compatible with an enhanced level of gluconeogenesis in type III disease.

The concentration of glycogen in the liver is markedly elevated. The amounts vary from 15 to 21 percent [10, 35]. Normal liver has less than 6 percent glycogen. Concentrations in muscle are less, approximating 6 percent in patients with type III disease and less than 1.5 percent in controls. Accumulation of glycogen of abnormal structure has also been reported in erythrocytes [43].

Depressed levels of enzymes other than the debranching enzyme are sometimes found in biopsies of these patients. For instance, glucose-6-phosphatase activities are sometimes somewhat low. That these are secondary effects has been suggested by the successful induction of increase to normal activity by the administration of triamcinolone [44].

GENETICS AND PATHOGENESIS

The disorder is transmitted by an autosomal recessive gene. Heterozygote detection has been carried out by the assay of debrancher enzyme activity in leukocytes and erythrocytes; intermediate levels were obtained [12, 45–47]. In families in which the patient lacks immunoprecipitable debrancher protein, carrier detection can be accomplished by Western blot [48].

The disorder has been recognized to be relatively high in frequency in Israel [12], where it makes up 73 percent of patients with glycogen storage disease. All of these are Sephardic Jews of North African origin, in whom the incidence figure was estimated at one in 5420. Gene frequency also appears to be high in the Faroe Islands [49]. Prenatal diagnosis has been accomplished by the study of the enzyme activity of immunoblot of cultured amniocytes and chorionic villus cells [50–52], but the assay of the

enzyme is demanding, because activity is so low in these materials and immunoassay is only useful in families in which patients have no enzyme protein.

The molecular defect is in the activity of amylo-1,6-glucosidase (Figure 61.1) [4, 5, 53, 54]. A number of different methods has been used to assay the enzyme. The overall reaction catalyzes the production of glucose from phosphorylase limit dextrin. The partial reactions, transferase and glucosidase, appear to reside on a single polypeptide chain [55]. Enzyme deficiency has been demonstrated in leukocytes [45, 50, 56, 57], erythrocytes [58, 59], and cultured fibroblasts [60], as well as liver and muscle [4, 5, 53, 54]. In most patients, there is parallel reduction in all tissues tested, regardless of the assay method employed. However, there are some discrepancies, and it is clear that a few patients with proven hepatic deficiency of the enzyme have had normal activity in muscle, leukocytes, erythrocytes, or fibroblasts [6, 46, 48, 61–63]. This means that the diagnosis cannot be excluded without assay of the liver or mutational analysis. It also seems likely that myopathy would only be expected in those with abnormal enzyme in muscle, and this seems to be the case [29]. The most common situation in which activities of both glucosidase and transferase is deficient in both liver and muscle is sometimes referred to as IIIa; when the deficiency of both activities is found only in liver, it is referred to as IIIb; and the instances in which there is selective loss of only the glucosidase activity or only the transferase activity, have been called IIIc and IIId, respectively [48, 54].

Studies using antibody to the normal debranching enzyme have revealed absence or considerable reduction of cross-reacting material (CRM) [48, 64]. The amounts of protein do not correlate with clinical severity [65].

The gene on chromosome 1p21 is in the area to which amylase genes have been mapped [7]. A variety of different mutations is responsible for this disease [6]. The isolation of the gene and determination of its sequence [6, 66] has elucidated a structure of 85 kb with 35 exons. The protein is a large monomeric structure of approximately 165 kDa. There is a single gene in liver and muscle, and the coding sequences of the two mRNAs are the same and code for a protein of 1532 amino acids. Isoforms differing in the untranslated region appear to account for tissue differences.

Determination of the nature of mutation has provided correlations between molecular abnormality and clinical phenotype. In a patient with quite severe IIIa disease, an apparently homozygous mutation was found in which a single base (A4529) was inserted, changing a tyrosine to a stop codon at amino acid 1510 [67, 68]. Two mutations in exon 3 at amino acid codon 6, 17delAG and Q6X, were found in three patients with type IIIb and not in type IIIa. Two patients had deletion of AG at nucleotides 17 and 18, leading to a truncated protein. The third had a C-to-T change at nucleotide 6, which changed glutamine to a stop. These mutations were not found in 31 patients with IIIa, two with IIId, or 28 controls. DNA diagnosis can be made on a blood sample in patients suspected of having type IIIb disease. A deletion, 4455delT, was found in all of the Sephardic Jewish patients reported [69]. A donor splice-site mutation was found in a Japanese patient [70].

It has been observed that type IIIb patients have mutations in exon 3, while downstream mutations were found in IIIa patients [71]. In a series of 44 patients from Italy [15], two mutations were found in 69 percent, only one allelele in 14 percent, and no mutations in 17 percent. Most mutations were null alleles. The IVS 21 + 1G/A intronic change was the most frequent (23 percent). Patients with null mutations tended to have more severe myopathic or hepatic disease.

Heterozygous expression of myopathy manifested by exercise-induced myalgia and weakness was reported in a large family with a truncating mutation, p.W1327X [72].

A novel missense mutation p.R1147G was found in a Turkish study [73]. There were six nonsense mutations in this series. Mutations causing premature termination continue to be common in a variety of ethnic populations [74]. Genotype was reported to correlate for two mutations, one 3964 del T with severe, early onset symptoms, and the other an A to G transition at −12 upstream (IVS32-12A > G) associated with mild IIIb disease in quite different ethnic backgrounds [75]. In 43 Chinese patients, 51 different mutations were identified [76]. The most common was c.1735 + 1G > T. In some patients with missense or small inframe deletions, normal CK level was observed.

TREATMENT

Frequent high-carbohydrate feedings in infancy and early childhood are often all that is necessary for the management of the hypoglycemia of glycogenosis III. Cornstarch supplementation has facilitated this regimen, particularly if used at bedtime or during infant feedings at night [77–79]. Few, if any patients, need nocturnal enterogastric infusion, which may be dangerous if the tube becomes disconnected in the middle of the night. Serious, permanent brain damage may ensue. There is no need to restrict fructose and galactose in this disease.

A diet high in protein has been advocated for patients with this disease, but it has been our experience that pediatric patients will seldom eat a diet high enough in protein to make an appreciable difference in any of the abnormalities we could measure. Added protein has been obtained by mixing supplemental cornstarch with yogurt and other products [79].

The enhanced gluconeogenesis in this condition makes the use of high-protein feedings logical [41]. Diets in which 20–25 percent of the calories are from protein and only 40–50 percent from carbohydrate have been employed. One approach has been to give between one quarter and one third of the calories as nocturnal enteral therapy high in protein. This has been recommended for patients with myopathy or growth impairment [80, 81]. Patients were reported to experience improved muscle performance, in

some cases quite dramatic. In others, effects were minimal or temporary [27]. In younger patients, there was improvement in growth [68, 79]. An older patient who discontinued the regimen experienced a recurrence of weakness that did not remit when therapy was resumed. Dramatic improvement in cardiomyopathy was reported [82] in a 22-year-old man on increasing the protein content of his diet to 30 percent of total calories.

In asymptomatic patients in childhood who nevertheless had elevated levels of CK and transaminases in the blood, we found no effects of cornstarch or a high-protein diet on these abnormalities. In contrast, we reasoned that if muscle were being broken down to provide alanine for gluconeogenesis, the provision of supplemental alanine might be therapeutic. We have observed a considerable improvement in levels of CK and transaminases [83]. Doses of alanine have ranged from 0.25 to 2.0 g/kg per day. A teaspoonful of alanine weighs 3.78 g.

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PART 7

PEROXISOMAL DISORDERS

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62

Adrenoleukodystrophy

MAJOR PHENOTYPIC EXPRESSION

X-linked cerebral demyelinating disease with onset in males in childhood, usually with behavioral abnormalities progressive to dementia, speech difficulty, and loss of vision and hearing; relentless progression to decorticate spastic quadriparesis; pigmentation of the skin; adrenal insufficiency; cytoplasmic inclusions; accumulation of very long-chain fatty acids, particularly hexacosanoate (c26:0); defective activity of very long-chain acylCoA synthetase; and mutations in the adenosine triphosphate (ATP)-binding cassette (*ABCD1*) gene for peroxisomal transmembrane transporter, adrenoleukodystrophy (ALDP) protein.

INTRODUCTION

Adrenoleukodystrophy (ALD) is a progressive cerebral degenerative disorder with onset in childhood, in which there is increased pigmentation of the skin and laboratory evidence of degenerative disease of the adrenals [1, 2]. The disease appears to have been first described in 1910 by Haberfeld and Spieler [3], and the neuropathologic findings by Schilder [4]. In 1923, Siemerling and Creutzfeld [5] were the first to put together the adrenal and cerebral disease in the definitive description; they referred to it as a bronzed disease in which there was sclerosis and encephalomyelitis, and it has been referred to as a bronzed Schilder disease, but in spite of the pigment, which may serve as the alerting sign to the diagnosis, most patients have full progression of the cerebral manifestations without the clinical symptomatology of adrenal insufficiency [2, 6, 7]. The lipid inclusions in the adrenal were first recognized by Schaumberg and colleagues [6, 7] who found that they were composed of cholesterol esters [8]. The term "adrenoleukodystrophy" was first employed by Blaw [9]. This disease appears to be the cause of Schilder disease in most males [9].

Neonatal adrenoleukodystrophy (Chapter 63) is a very different disease with an entirely different phenotype and an autosomal recessive, as opposed to X-linked transmission.

The X-linked nature of ALD was first recognized by Fanconi and colleagues [10]. A more indolent, slowly progressive phenotype with onset even in adulthood was described in 1976 [11], and this has been referred to as adrenomyeloneuropathy [12]. Despite the slower course, characterized mainly by progressive spastic paraparesis, this is fundamentally the same disorder, and the two phenotypes have been observed in siblings [13]. The disease expresses in a portion of heterozygotes, in whom the picture is of adrenomyeloneuropathy.

The cholesterol esters found in the adrenal glands contain large amounts of very long-chain fatty acids (VLCFA) [8]. Moser and colleagues [14, 15] found that these elevated VLCFA could be demonstrated in blood and cultured fibroblasts, and this has become the method of choice for diagnosis. They can be demonstrated by gas chromatography and gas chromatography-mass spectrometry (GCMS). The oxidation of VLCFA takes place in peroxisomes. The enzyme that catalyzes the formation of the CoA esters of these VLCFAs is defective in this disorder (Figure 62.1) [16, 17]. However, the defective gene is that of

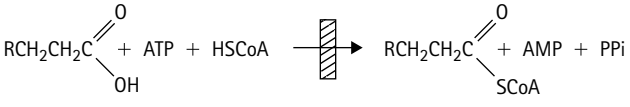

Figure 62.1 Very long-chain acylCoA synthetase (VLCAS), the activity is defective in ALD.

a peroxisomal membrane transporter protein [18]. The gene was mapped to Xq28 [19]. It was isolated and found to be a member of the ABC transporter family [18, 20]. Of more than 200 mutations identified, approximately 50 percent were missense and 24 percent frameshifts; large deletions, insertions, and splicing defects are uncommon [21–23]. The gene is now referred to as *ABCD1* and its product as ALDP.

CLINICAL ABNORMALITIES

The presenting findings in ALD are often behavioral (Figures 62.2 and 62.3) [2, 9, 24]. By four to eight years of age, some patients, who have passed early milestones normally, begin to be hyperactive and withdrawn. Others may be aggressive or belligerent, and this behavior may occur in bizarre outbursts. Initial referral is often to a psychiatrist or psychologist. Poor school performance may be another presenting problem, but often there is inattentiveness and poor concentration. Diagnosis of attention deficit disorder and treatment with stimulants, such as Ritalin, is a common history. There may be changes in, or failing, memory. Difficulty in communication or loss of acquired skills in speech is commonly encountered. This is the classic, or childhood cerebral onset phenotype, that occurs in approximately 40 percent of patients. In a small number of these patients, onset is in adolescence, but most begin by three to ten years [2, 8, 24]. Despite differences in phenotype, even in the same family, it is clear that this is a progressive disease [25].

Visual disturbances are common in these patients and may occur early. Homonymous hemianopsia has been observed in at least two patients [2], and there may be a striking loss in visual recognition of objects. There may be a transient horizontal nystagmus in the early stages of visual loss. There may be strabismus or double vision. Ultimately, virtually all patients have a loss of vision as a prominent feature. Optic atrophy is usually a late finding, but rarely it may be seen early. The pupillary response to light remains intact until late in the illness. Hearing loss is also characteristic and may occasionally be seen as an early finding. Difficulty in understanding speech in a noisy room or over the telephone may be an early sign of impaired auditory discrimination. In testing patients at risk, the earliest manifestations may be detected by neuropsychometric testing for abnormalities in visual or auditory processing, new learning or short-term visual memory [26].

Figure 62.2 Two children with ALD. The diagnosis was made at six and eight years of age, respectively. Onset was with attention deficit and hyperactivity followed by loss of verbal skills. Hyperpigmentation was particularly prominent in inguinal areas.

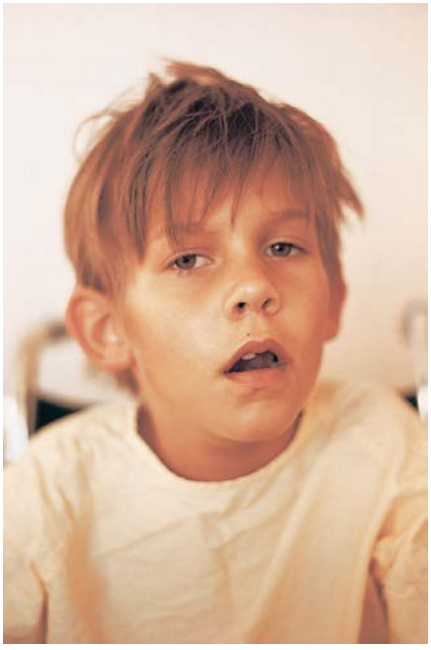

Figure 62.3 Eight-year-old with adrenoleukodystrophy. The blank open-mouthed expression was characteristic. By nine years of age, he had spasticity, was blind, and had multiple contractures.

Abnormalities in gait may be seen early. Characteristically, the gait is stiff-legged and unsteady. Deep tendon reflexes may be increased. Asymmetry of relatively early findings may confuse the diagnosis. A number of patients have had hemiparesis, but eventually they all develop spastic quadriparesis. Astereognosis, graphesthesia, and apraxia may be seen early. Dysarthria and dysphagia occur regularly. Once they appear, neurologic manifestations are rapidly progressive, usually over months or years, to a decorticate state in which the patient is blind and deaf. Seizures are relatively common late in the disease and may be focal or generalized. In some patients, they may occur early, even as the first neurologic feature. In a series of 167 patients, the duration of disease from the onset of neurologic manifestations until death was 1.9 ± 2; range was 0.5–10.5 years [24]. Behavioral change may be the initial

clue to cerebral demyelination [26]. The disease may appear to be psychiatric, but it is ultimately progressive.

The brown pigmentation may be evident at the onset of symptoms, but it is usually found later. It occurs particularly in areas not exposed to the sun, such as skin folds, inguinal areas, areolas, and the buccal mucosa. Clinical signs of adrenal insufficiency are seen in some patients and have antedated the neurologic manifestations in some patients [2]. For this reason, it is worth a specific assessment for ALD in any boy who develops adrenal insufficiency during childhood. The onset of adrenal failure is usually insidious, with fatigue and intermittent vomiting. Two patients have been reported who had arterial hypotension, but it is easy to see how the other manifestations of adrenal failure could be missed in a patient with advanced neurologic disease. The most useful test of adrenal function is to assess the response to adrenocorticotropin (ACTH) [24]. Levels of cortisol in plasma or urinary 17-hydroxy steroids may be low, but more important is a failure to respond to ACTH. Testing with metyrapone early in the illness, when the ACTH stimulation test may be normal, reveals normal pituitary function. Concentrations of electrolytes in serum have regularly been normal. Plasma levels of ACTH may be increased.

Among variant presentations, patients with adrenomyeloneuropathy were so characterized because of prominent spinal cord involvement [11, 12]. Initial symptoms of stiffness or clumsiness in the legs progress to spastic paraplegia. Generalized weakness, loss of weight, hyperpigmentation, and attacks of vomiting are signs of adrenal insufficiency. The neurologic disease often progresses slowly over five to 15 years. The patient becomes wheelchair-bound and may develop problems with urination. Somatosensory and brainstem auditory evoked responses are abnormal. Cognitive function is abnormal in about half of patients. Vibration sense in the lower extremities may be impaired and so may nerve conduction velocity. Impotence is common [27]. Ultimately, gonadal insufficiency is common [28].

Symptoms in the female heterozygote may resemble those of adrenomyeloneuropathy. In a few, there is severe disability with paraparesis [29, 30]. These women may be thought to have multiple sclerosis, if there is no family history of involved males [31]. One patient had intermittent paresthesia from the age of 40 years [31]. Some asymptomatic patients have had hyperreflexia and impaired vibration sensation in the legs. Some have been diagnosed only after they had an affected son [31]. Adrenal insufficiency is rare in most heterozygotes [32]. Some have had dementia. Others have had an adolescent onset of the kind of progressive cerebral disease seen in the male, even with adrenal insufficiency [30, 33].

Rarely, males have had an adult onset of cerebral disease without cord involvement [29]. Some have been thought to have schizophrenia, or Kluver-Bucy syndrome [34]. Psychotic symptoms in a patient with Addison disease should trigger this diagnosis, but adrenal function may be normal.

A small number of male patients, mostly from Japan, has presented with a picture of olivopontocerebellar atrophy [35–37]. Most were adults. A five-year-old Japanese child presented with cerebellar ataxia [38]. Imaging revealed cerebellar and pontine atrophy. The disease was progressive.

At the other end of the spectrum, three infants have been reported [39], whose phenotype was that of a peroxisomal disorder, such as neonatal ALD (Chapter 63). They had profound hypotonia, failure to thrive, and cholestatic hepatic disease. Two had seizures and the third episodic opisthotonus. In a single autopsy, the adrenals were small and fibrotic.

Some patients with this disease have had pure Addison disease without neurologic findings [39, 40]. In areas in which adrenal tuberculosis is rare, this disorder may represent a significant proportion of patients with Addison disease. Some patients, found by testing relatives of known patients, have been asymptomatic for long periods of time, but it is expected that sooner or later they will all develop neurologic abnormalities. Some have developed prominent cerebellar signs or a picture of olivopontocerebellar degeneration [35–37, 41].

Neuroimaging by computed tomography (CT) or magnetic resonance imaging (MRI) [42–44] reveals evidence of leukodystrophy in the cerebral white matter (Figures 62.4–62.6). Temporal and parieto-occipital involvement are seen most frequently, but there may be widespread involvement, including the cerebellar white matter and the corticospinal tracts. Some patients have had cerebral atrophy. There are widespread symmetric,

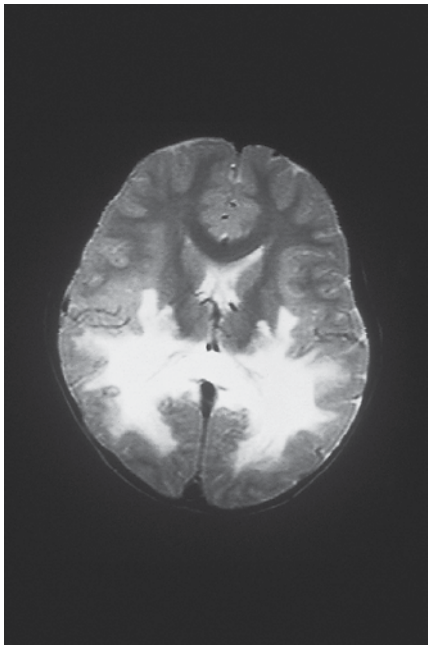

Figure 62.4 MRI of the brain of GQ, a seven-year-old boy with ALD. He had experienced the sudden onset of seizures after a period of disturbed behavior. He had pyramidal tract signs and spatial agnosia. Imaging revealed the typical white matter disease.

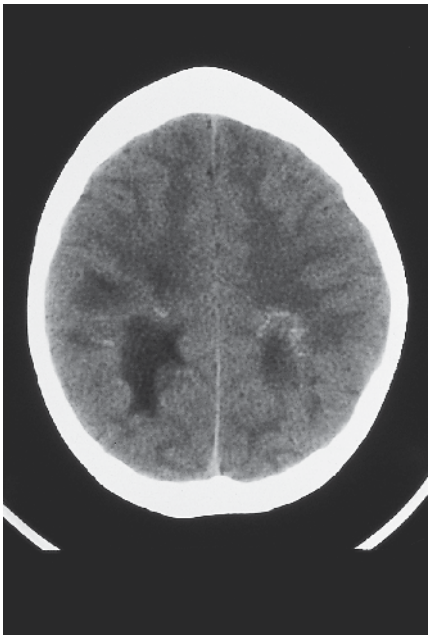

Figure 62.5 CT scan of the brain of HM, a boy with ALD. There was extensive leukodystrophy and calcification around the lucent area of demyelination.

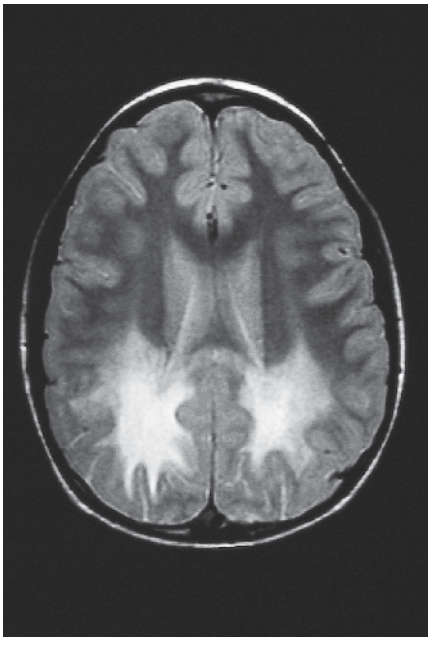

Figure 62.6 MRI of the brain of HM. The intense T_2 signal in the white matter was indicative of the leukodystrophy.

confluent, low-density lesions on CT or T_1-weighted MRI or increased density on T_2 in the periventricular white matter of the parieto-occipital areas that enhance anteriorly in the CT on infusion of contrast material. Repeated scans over time show a caudal–rostral progression of the demyelination. The enhancement with contrast reflects breakdown of the blood–brain barrier, and this is seen also on brain scintiscan, which shows increased uptake in the involved areas. In some patients, the lesions found on imaging the white matter have been the first clue to the diagnosis. In a few patients, atypical unilateral lesions have, along with (in one patient) symptomatology of unilateral headache, visual loss, weakness, and hyperreflexia, led to a diagnosis of brain tumor [45]. Biopsy revealed leukodystrophy, and that suggested the diagnosis. In asymptomatic patients at a mean age of 6.7 years, MRIs were normal and psychologic testing revealed normal cognitive function [46]. In 56 adult patients with white matter abnormalities, 42 had corticospinal disease, and 50 percent of these had progression of lesions over three to five years, but disease progression was slower than in affected children [47].

Brainstem auditory evoked responses in ALD may have abnormal [48] asymmetry in that wave VI on one side may be absent. There may be progressive loss so that ultimately only a prolonged wave I is recordable bilaterally [49]. Electroencephalograms (EEG) are usually abnormal, most commonly showing diffuse slow activity or large-amplitude slow waves over the posterior regions [50].

Pathologic examination of the nervous system reveals extensive diffuse demyelination in the cerebral white matter, most prominent in the occipital and posterior parietal areas and spreading in a caudal–rostral direction [2, 51, 52]. There is secondary loss of axons and gliosis. Late findings may be cavitation or calcification. In addition, there are diffuse perivascular infiltrations of lymphocytes. These inflammatory findings are not seen in adrenomyeloneuropathy, which is predominantly a distal axonopathy [52]. In the adrenal gland, there are ballooned cortical cells, which have characteristic striations [7].

The nature of the disorder was originally clarified by the finding of characteristic cytoplasmic inclusions in large glial cells or macrophages of the central nervous system (CNS) and in adrenal cortical cells [51, 53]. The inclusions in the CNS stain positive with periodic acid-Schiff (PAS) and Oil Red O stains. Sudanophilia is common, but may be absent. The electron microscope reveals the pathognomonic ultrastructure of curvilinear spicules with central lucent spaces (Figures 62.7 and 62.8) [51, 53]. Similar inclusions have been seen in Schwann cells [54], making the diagnosis possible by sural nerve biopsy, but this has more often been negative [2]. Testicular tissue may show identical ultrastructural lamellar lesions in Leyden cells [55]. Characteristic ultrastructural lesions have been seen in Schwann cells obtained by conjunctival biopsy, and vacuoles have been seen in eccrine glands of the skin [56]. Chemical methods have largely supplanted biopsy approaches to diagnosis.

GENETICS AND PATHOGENESIS

Adrenoleukodystrophy is an X-linked disorder that is not fully recessive in its expression, as there may be clinical expression in female heterozygotes.

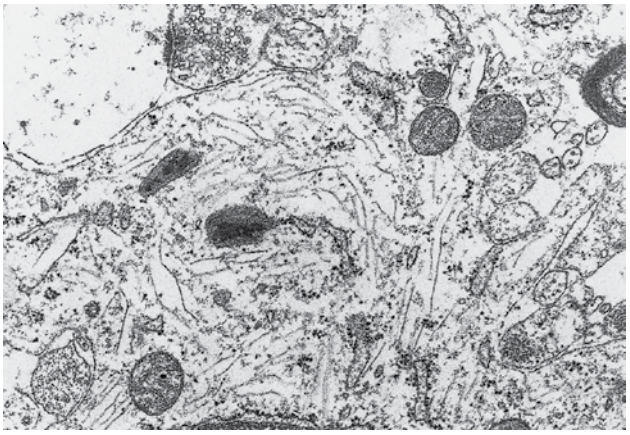

Figure 62.7 Electron microscopic section of the brain, illustrating the characteristic fine thin spicules. These cytoplasmic inclusions may be straight or curved and contain a central electrolucent space bound by a thin electrodense membrane. (Illustration kindly provided by Dr Henry Powell of the University of California, San Diego.)

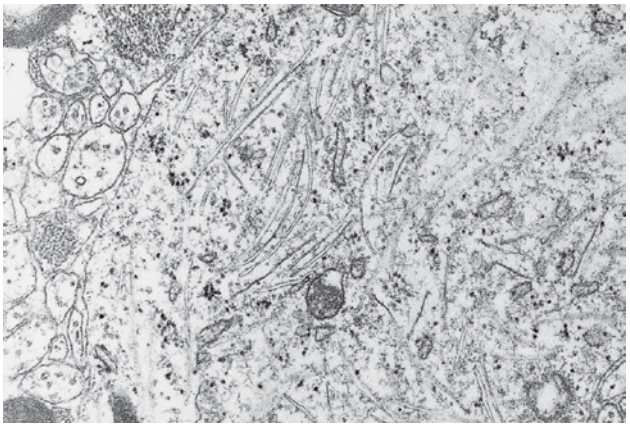

Figure 62.8 Cytoplasm of this brain cell illustrates the characteristic scimitar-shaped inclusions. (Illustration kindly provided by Dr Henry Powell of the University of California, San Diego.)

The specific biochemical abnormality in ALD is the accumulation of very long-chain unbranched fatty acids, which are saturated or mono-unsaturated. The carbon lengths of these compounds are 24 to 30. They are found normally among the fatty acids of the cholesterol esters and gangliosides of the cerebral white matter and the adrenal cortex, and C26:0 makes up as much as 5 percent of the total fatty acids of cerebrosides and sulfatides of the normal brain [8, 57–59]. Similarly, the VLCFA that accumulate in ALD are predominantly those with a chain length of 26 [15]. They are largely hexacosanoic acid (C26:0) (cerotic acid). Accumulation of these VLCFA has been demonstrated in cultured fibroblasts [14, 59, 60] and muscle cells [61]. In cultured fibroblasts, the ratio of C26 to C22 fatty acids has been useful in diagnosis, as well as the level of C26:0 [14]. The ratio was 0.76 in six patients with clinically typical disease and 0.78 in five patients with autopsy-proven disease, while in controls it was 0.06. The concentrations of these same very long-chain saturated fatty acids in plasma are also increased [62], and this is the most convenient method for definitive diagnosis. The levels of C24 (lignoceric acid), C25, and C26 are significantly elevated, while those of C20 and C22 are normal. The C26:C22 ratios of hemizygotes are approximately five times those of controls. In general, the plasma assay is sufficient for diagnosis. In instances in which the data are equivocal, the fibroblast assay is employed. The accumulation of VLCFA in patients with adrenomyeloneuropathy is no different than in patients with classic ALD [14]. False positives may be caused by hemolysis in the sample, a ketogenic diet, and peanut butter.

The accumulation of VLCFA in ALD is a consequence of abnormality in the oxidation of these VLCFA, which takes place in peroxisomes [63]. Studies of oxidation to $^{14}CO_2$ of ^{14}C-labeled fatty acids in fibroblasts revealed impaired production of CO_2 in ALD [64]. Oxidation of hexacosanoic (C26:0) acid was 14 percent of control, and that of lignoceric (tetracosanoic) (C24:0) was 8 percent of control.

Heterozygotes can be detected by assay of the VLCFA of plasma or cultured fibroblasts [65]. Levels in both plasma and fibroblasts, especially using the ratios of C26:C22 or C24:C22, were intermediate between patients and controls, and significantly different from the latter, but there was a small amount of overlap. By assaying both fibroblasts and plasma, over 90 percent of obligate heterozygotes can be identified [65]. Cloning of fibroblasts from heterozygotes yielded two populations of cells, one normal and the other identical to patients with ALD in its C26 fatty acid content [65]. These experiments proved that the gene is on the X chromosome and that it is subject to inactivation. Studies of women doubly heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD) revealed close linkage between the two loci [65].

The gene for G-6-PD has been mapped to Xq28 [66], localizing the gene for ALD to this locus of the X chromosome. This has led to the exploration of DNA probes, and DXS52 was found to be closely linked to ALD [67]. The linkage can be used in heterozygote detection [68] and effectively resolves any situations in which the assay of VLCFA is not clear. Mutation analysis is the most reliable method for detection of heterozygosity [69].

The linkage to DXS52 has also been used successfully in prenatal diagnosis [70]. The assay for VLCFA is usually employed as the procedure of choice for prenatal identification of the affected male fetus [71]. Cultured amniocytes and chorionic villus material have been used, but normal VLCFA have been found in cultured chorionic villus cells in pregnancies where the fetus turned out to be affected [72, 73]. LC-MS/MS for 26:0 lysophophatidylcholine can be used for prenatal diagnosis of the X-linked disorder with simultaneous analysis of acylcarnitines [74].

Oxidation of C26:0 fatty acid in cultured chorionic villus material has been employed for prenatal diagnosis [75]. In terminated pregnancies, fetal ultrastructural as

well as chemical abnormalities were clearly present. When the mutation is known, it provides a definitive method of prenatal diagnosis [76,77]. Newborn screening has been successfully employed [78].

Defective oxidation of VLCFA in this disease is a consequence of failure to form the coenzyme A esters. The synthetase enzyme (see Figure 62.1) whose activity is defective has been localized to the peroxisome [79–81].

The gene for ALD was found by positional cloning within the Xq28 region [18]. It spans approximately 20 kb in ten exons. The deduced amino acid sequence placed it among the ATP-binding cassette superfamily of transmembrane transporter proteins. The structure has extensive similarities to the 70 kDa peroxisomal membrane protein, PXMP1 [18]. A few deletions in the gene were identified in patients by Southern blot analysis [18]. The majority of the mutations are missense, and a majority of all mutations are unique to the family in which they are found [23]. Mutations have clustered in the membrane spanning region and in the nucleotide binding region, and there is a hot spot in exon 5. Correlation between genotype and phenotype has not been possible. Polymorphism has also been identified (N13T) which caused amino acid change, but does not affect function of the ALDP [21]. In the three patients with neonatal presentations suggestive of peroxisomal disease [39], there was no immunochemically deflectable ALDP, and large deletions were found in the *ABCD1* promoter region and the adjacent *DXS1357E* gene. Deletions in this latter gene cause creatine deficiency in brain as a result of deficiency in the X-linked creatine transporter gene (*SLC6A8*) [82]. Among 112 patients with adrenomyeloneuropathy, mutations were found in all of the probands [83]. They were scattered over the gene and did not correlate with phenotype. About 50 percent were missense mutations, of which 64 percent were at CpG dinucleotides. In a more recent review of mutations, the majority were point mutations [84]. Among the heterozygotes, the only reliable method of detection was mutation analysis; tests of VLCFA in plasma were unreliable. In a three-year-old Japanese boy, a deletion of exons 3–10 of the *ABCD1* gene was fused to a neighboring gene, *PLNXB3* [85].

It is not clear how the defect in the gene affects VLCFA synthetase activity, and ALDP does not function as a synthetase. Nevertheless, it appears likely that the disease in the CNS and in the adrenal results from the accumulation of VLCFA [86, 87].

A novel donor site mutation (c.1272 + 1g > α) was reported [88] which led to a 121 bp deletion and a stop codon.

In studies of expression of the gene in tissues of patients, correlations with phenotype were elusive [89]. Accumulation of saturated VLCFA in white matter did correlate with phenotype.

Cloning of individual fibroblasts of heterozygotes demonstrated two populations of cells consistent with the Lyon hypothesis [90]. The content of C26 fatty acids was employed as the marker. There were more mutant clones than wild type, consistent with the fact that heterozygotes express disease phenotypes and a selective advantage of mutant cells. This contrasts with Lesch-Nyhan disease in which there is selection for wild type in females.

TREATMENT

The course of ALD has usually been relentless, and no therapeutic measures appear to be effective. Symptomatic therapy is important [91], and support groups may be helpful to families. Physiologic amounts of adrenal steroid replacement therapy are effective in the management of the adrenal disease.

Bone marrow transplantation has been carried out without improvement in neurologic status, but encouraging results have been obtained in patients treated early in their courses [92–99]. It is reported that some patients have become clinically stable after transplantation, and some have even improved. It is clear that the window is small, and there is an inverse correlation between severity of the manifestation at transplant and outcome [94]. In patients studied with proton magnetic resonance spectroscopy, transplanted patients fell midway between controls and untreated patients in the ratios of N-acetylaspartate (NAA) to creatinine and NAA to choline [97]. Criteria for transplantation remain unclear but, increasingly, patients have transplanted for worsening MRI before clinical regression.

Reduction in NAA and increase in choline on the magnetic resonance spectroscopy have been used as criteria for transplantation. There is nevertheless increasing evidence [95, 96] that best results have come from early transplantation.

Gene therapy has been reported in two patients [99]. Autologous cells removed from the patients were transfected with a virus vector containing wild-type *ABCD1*. Over three months, there was polyclonal restitution in hematopoietic cells and T lymphocytes expressing the ALD protein and a cessation of cerebral demyelination. A larger experience from France [100] reported resolution of some neurologic abnormalities but plasma VLCFA remained elevated.

Studies using deuterium-labeled hexacosanoic acid indicated that a substantial amount of the C26 fatty acids in the brain is of dietary origin [101]. Although there is also evidence that fibroblasts of patients are able, unlike controls, to synthesize C26 fatty acid from stearic acid [101], these observations have raised the possibility of dietary therapy. Restriction of the intake of VLCFA has been undertaken in this disease without effect on levels of VLCFA or clinical course. The observation [102] that the addition of mono-unsaturated fatty acids, such as oleic acid to cultured fibroblasts of patients, leads to reduction in accumulation of VLCFA, led to the use of glyceryltrioleate in therapy. Glyceryltrierucate was even more effective *in vitro*, and this has led to the development of Lorenzo's oil, a 4:1 mixture of trioleate and trierucate oils, named after the patient whose

parents popularized it, as shown in the film, "Lorenzo's Oil". Treatment does bring plasma levels of C26:0 to normal, but it is clear that the neurologic progression of the disease is not halted. Double blind placebo-controlled studies have not been done, but Lorenzo's oil does not appear to be useful in patients who have demonstrated neurologic regression [103, 104]. It may be worth exploring in patients with adrenomyeloneuropathy, but the evidence is against it [105]. Many patients develop thrombocytopenia [106] and so platelet counts must be monitored. Levels of essential fatty acids should be monitored to prevent deficiency. In Moser's 2005 publication [107], 89 presymptomatic boys identified were treated with Lorenzo's oil and moderate restriction of the intake of fat. In a short follow up (6.9 ± 2.7 years), 29 percent developed abnormalities on MRI and 11 percent neurologic abnormalities. He recommended oil therapy in boys with normal MRI.

By analogy with its beneficial effect in sickle cell anemia, where they increase fetal hemoglobin, butyrate and 4-phenylbutyrate have been explored in ALD. Cultured cells from patients were found to have improvement in the oxidation of VLCFA, and amounts of stored VLCFA in the brain of a mouse model were decreased by exposure to phenylbutyrate [108]. Preliminary studies in man are said to be underway.

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63

Neonatal adrenoleukodystrophy/disorders of peroxisomal biogenesis

MAJOR PHENOTYPIC EXPRESSION

Profound hypotonia, seizure disorder, hepatic fibrosis, atrophic adrenals, accumulation of very long-chain saturated fatty acids, pipecolic aciduria, and defective biogenesis of peroxisomes as a result of failure to import peroxisomal proteins.

INTRODUCTION

Neonatal adrenoleukodystrophy (ALD) was first described in 1978 by Ulrich and colleagues [1]. A relatively small number of patients has since been recognized [2–12]. Accumulation of very long-chain fatty acids (VLCFA) in this condition is indicative of multiple defective peroxisomal functions. This disorder, infantile Refsum disease, hyperpipecolic aciduria, and Zellweger syndrome fall into the same group of disorders of peroxisomal biogenesis, but mutations in at least 13 PEX genes have been identified, indicating fundamental defects in a number of steps in peroxisomal biogenesis [13–15]. Among them, Zellweger is the most severe, but neonatal ALD is also a very severe disease. No correlation between complementation group and phenotype has emerged. Zellweger syndrome is found in at least ten complementation groups and neonatal ALD in at least six of the same groups. Among patients with Zellweger syndrome, one has been shown to have a defect in peroxisomal assembly factor 1 (PAF1), a 35-kDa membrane protein involved in the assembly of peroxisomes [16]. Two patients were found to have mutated alleles in the 70-kDa peroxisomal membrane protein (PMP70) [17], which is a member of the multiple drug resistance-related adenosine triphosphate (ATP)-binding cassette transporter superfamily. Patients with defective biogenesis of peroxisomes have abnormality in virtually every peroxisomal function, notably the peroxisomal β-oxidation of fatty acids (Figure 63.1).

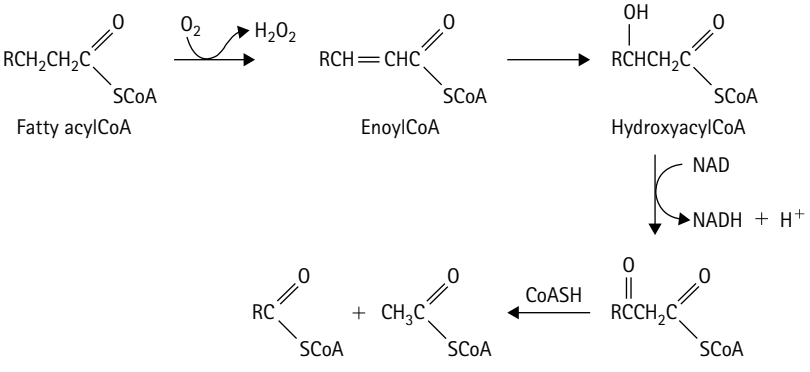

Figure 63.1 The pathway of carnitine-independent oxidation in peroxisomes. The NAD-dependent step and the one preceding it are analogous to mitochrondrial enzymes enoylCoA hydratase and 3-hydroxyacylCoA dehydrogenase and they are catalyzed by the bifunctional enzyme. This pathway catalyzes the metabolism of long- and medium-chain fatty acids, as well as the VLCFA. Short-chain acylCoAs and acetylCoA can then be transferred into carnitine esters via peroxisomal carnitine transferases.

Another peroxisomal assembly protein, PXR1, is the site of the defect in some patients with neonatal ALD. Two mutations have been identified: a nonsense and a missense mutation [18]. Genes for peroxisomal biogenesis in yeast have been extensively studied; they are referred to as PEX genes, and this usage has been adopted for the human orthologs [19]. Proteins that mediate the import of peroxisomal matrix proteins are called peroxins, and they are encoded by PEX genes. Members of the various complementation groups have mutations in what are now known as the human PEX genes. Thus, the mutations in the patient with neonatal ALD, referred to initially as PXR1, are in the PEX5 gene on chromosome 12p13. PEX1, the first PEX gene to be identified in yeast [20], situated on human chromosome 7q21-22, is the most common cause of neonatal ALD and Zellweger syndrome [21]. Heimler syndrome has recently been noted to be a relatively mild peroxisomal disorder with defects in PEX1 and PEX6 [22].

CLINICAL ABNORMALITIES

The clinical picture of neonatal ALD is dominated by extreme hypotonia and a severe convulsive disorder (Figures 63.2–63.5) [1, 3, 9]. The hypotonia may be evident on the initial neonatal examination and is severe enough to suggest a diagnosis of myopathy [9]. Cerebral manifestations are profound. Most of these infants show little evidence of psychomotor development. Sucking reflex is poor. They feed poorly and may fail to thrive

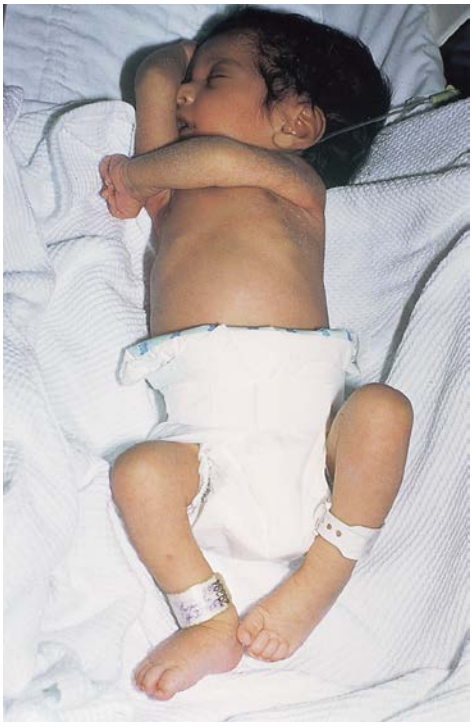

Figure 63.3 Baby girl M: A neonate with neonatal ALD who presented with severe neonatal seizures. She was extremely hypotonic. She trembled at slight touch. There was mild hepatomegaly and absent neonatal reflexes. MRI was consistent with abnormal myelination. Analysis of VLCFA revealed a C26 of 3.25 μg/mL and a C26/C22 ratio of 1.05. She died at three or four months of age at home.

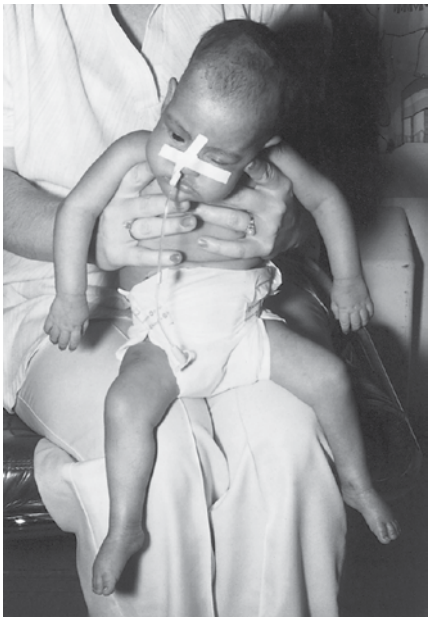

Figure 63.2 AA: At 3½ months of age, illustrating the hypotonia and the ptosis on the left. This patient had a peroxisome biogenesis disorder resulting from mutation T1467G in the PXR gene [18].

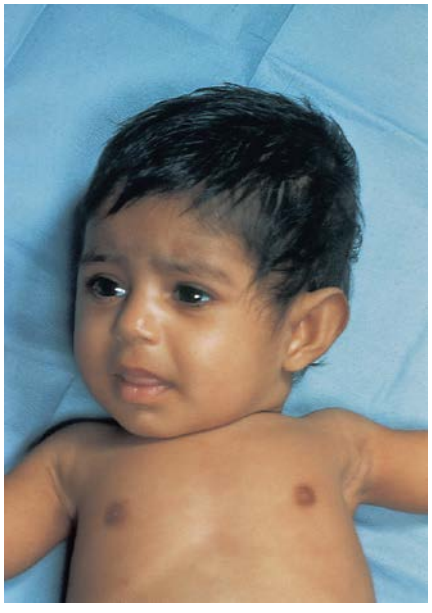

Figure 63.4 AH: A six-month-old boy who had severe early infantile myocolonic seizures. VLCFA were elevated. He died at one year of age.

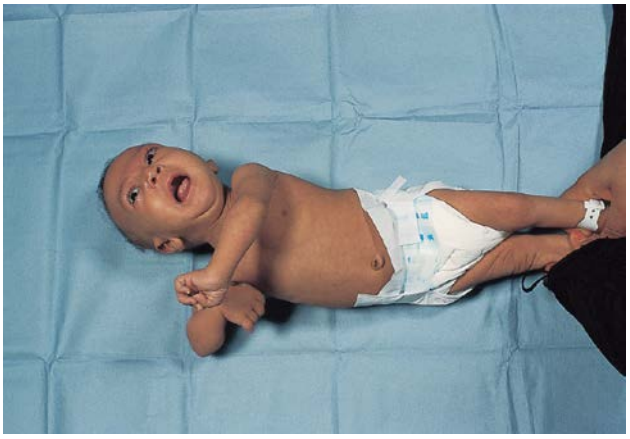

Figure 63.5 AH: The brother of the patient in Figure 63.4 at four months. He had uncontrollable early infantile seizures and elevated VLCFA. He died at ten months of age.

unless tube-fed. There are few or no spontaneous movements. The grasp and Moro responses are poor or absent. Tonic neck, stepping, and placing reactions are absent. Deep tendon reflexes are usually diminished. Two patients were reported to be macrocephalic [3]. Ocular abnormalities reported include nystagmus, optic atrophy, and pigmentary degeneration of the retina [1, 7, 22, 23].

Seizures usually begin within the first days of life and continue as a major problem. They tend to be refractory to anticonvulsant therapy. Seizures may be myoclonic, as well as grand mal. Shivering or trembling may be stimulated by light touch and may be reminiscent of autumn leaves. Electroencephalograms (EEG) are abnormal, usually showing multifocal spike discharges [1, 3]. In one patient, the pattern changed at one month to that of hypsarrhythmia [1]. Decreased nerve conduction has been reported [3]. The computed tomography (CT) scan may be normal, or show a mild decrease in white matter, or there may be many patchy lucencies on CT scan or magnetic resonance imaging (MRI) [3]. There may be enlargement of the ventricular system. Cerebrospinal fluid protein may be elevated [1, 3].

Hepatomegaly may be progressive [3]. Levels of transaminase activity in the blood may be elevated [3]. With time, there is little evidence of developmental progress. One patient could smile and roll from supine position at between seven and nine months, but lost these functions shortly thereafter. Another developed some lateral head movement and a smile, which she lost after 12 months [3]. One patient had a cataract [3]. Most patients have died before the second birthday [1, 3, 9]. Mean age of death of the patients was 15 months, while in classical Zellweger syndrome it was 5.7 months. In neonatal ALD, death has occurred as early as four months. A small number of patients have survived to teenage, albeit severely handicapped and dysmorphic, while some patients with infantile Refsum disease have reached adulthood [24, 25]. Impaired hearing and retinopathy have suggested a diagnosis of Usher syndrome. The mental age of patients has seldom exceeded 12 months and some have regressed at three to five years.

Dysmorphic features may be like those of Zellweger syndrome, but may be absent [9, 10]. Renal cysts are not found, nor is chondrodysplasia punctata. The typical facial appearance of the Zellweger syndrome includes a prominent high forehead and flat occiput with large fontanels and wide cranial sutures, abnormal helices of the ears, a broad nasal bridge, epicanthal folds, and hypoplastic supraorbital ridges (Figures 63.6–63.10) [26, 27]. In addition, there are hepatorenal abnormalities and stippled calcifications in the patellae. Nipples and external genitalia may be hypoplastic.

The advent of molecular understanding of the disorders of peroxisomal biogenesis may ultimately render the earlier distinct clinical phenotypes obsolete. It is clear that there is a spectrum from the very severe Zellweger phenotype to the severe neonatal ALD to the more indolent infantile Refsum disease, and that mutations in the same gene can produce any of these phenotypes. The infantile Refsum phenotype may include some dysmorphic features, such as epicanthal folds, a flat nasal bridge, and low set ears [28]. Hypotonia is impressive in all of these diseases [29]. Retinitis pigmentosa and sensorineural hearing loss are characteristic [30]. These patients learn to walk but with an ataxic gait and they are severely impaired [29]. Some patients have had normal intelligence [13].

The same phenotype can also be found in deficiencies of single peroxisomal enzymes (Figures 63.11–63.13). The enzymes include acylCoA oxidase-1 [31, 32], which phenotype has been referred to as pseudoneonatal ALD,

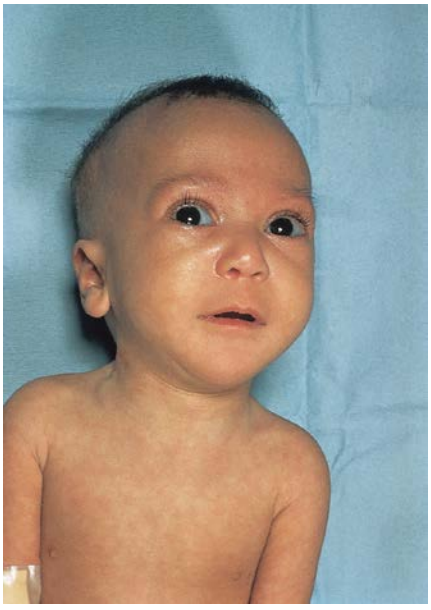

Figure 63.6 HS: A one-month-old girl with Zellweger syndrome, had the typical facial appearance along with severe hypotonia, absent development, and hepatomegaly. VLCFA were elevated. She died at four months of age.

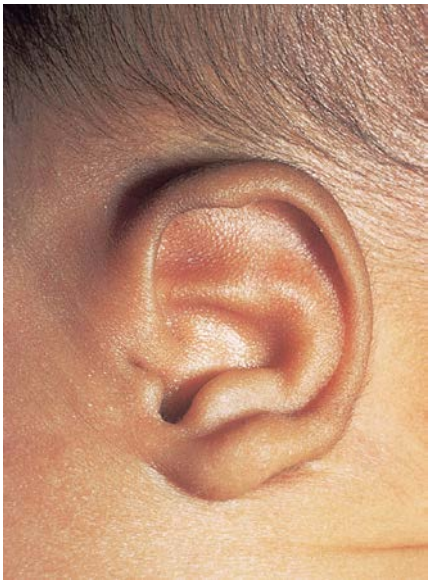

Figure 63.7 The ear of HS.

Figure 63.8 MB: An infant with Zellweger syndrome whose clinical and chemical presentation was like that of HS, but who was alive at 18 months when the family was lost to follow up. The forehead was striking.

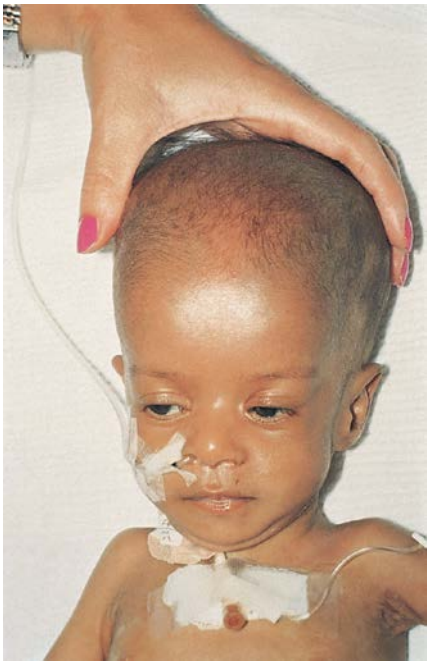

Figure 63.9 AM: A four-month-old infant with Zellweger syndrome and the typical facies. He had cataracts, hypotonia, and retinitis. The patellae were stippled. VLCFA were elevated, and there was pipeocolic aciduria. He died at six months of age.

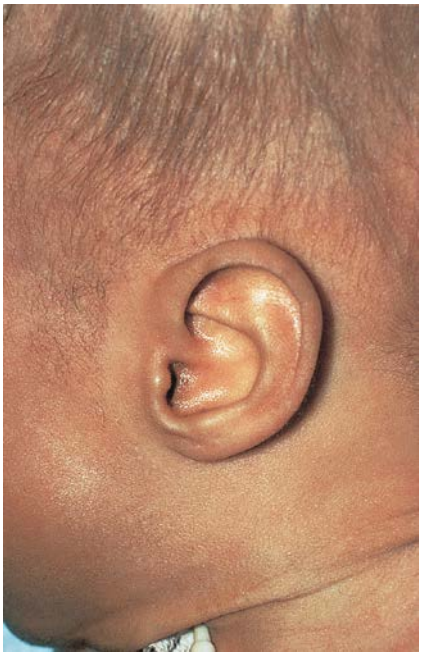

Figure 63.10 The ear of AM.

D-bifunctional protein [33, 34], and peroxisomal thiolase-1 [35], which phenotype was originally referred to as pseudo-Zellweger syndrome. In these disorders, VLCFA are elevated, but plasmalogen synthesis is normal. Among these disorders, deficiency of the D-bifunctional enzyme is much more common. This protein has both enoylCoA hydratase and 3-hydroxyacylCoA dehydrogenase activity. Among these patients, three subgroups have been identified [35, 36], one with deficient hydratase activity, one with deficient hydroxyacyl dehydrogenase activity, and one with absent protein and deficiency of both activities.

Patients with neonatal ALD usually have no clinical evidence of adrenal insufficiency. Electrolyte

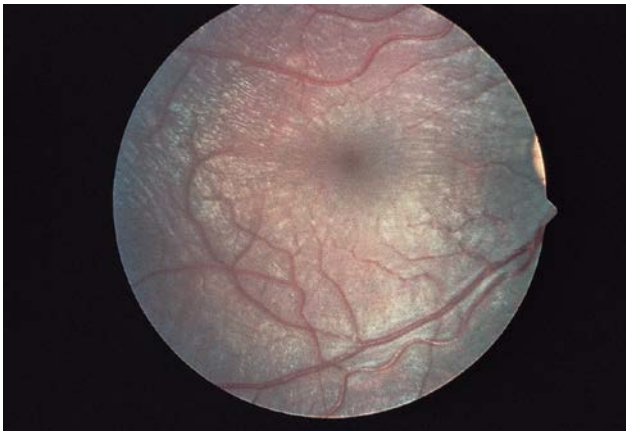

Figure 63.11 The tigroid retinitis pigmentosa of SM, a girl with peroxisomal bifunctional protein deficiency.

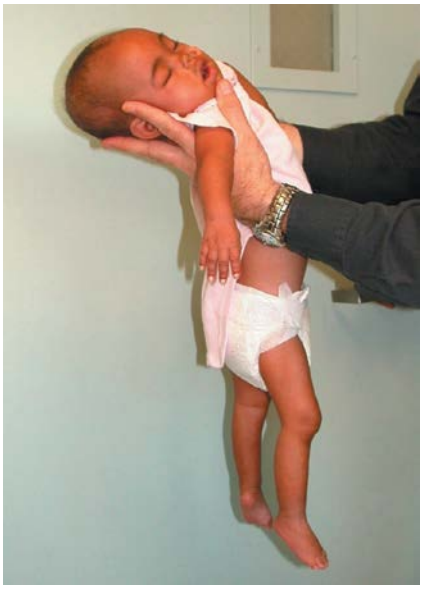

Figure 63.12 SE: A neonate with a typical neonatal ALD phenotype with intractable seizures and essentially no muscle tone. VLCFA were highly elevated, but plasmalogens were normal, indicating a single enzyme in peroxisomal fatty acid oxidation rather than a defect in peroxisomal biogenesis. A deficiency of the D-bifunctional protein was likely, but has not yet been tested. (Patient was kindly referred by Dr Keith Vaux.)

concentrations are normal. One patient had a low level of cortisol in the serum, but the cortisol response to adrenocorticotropin (ACTH) was normal. Most have had impaired cortisol responses to ACTH. One patient developed hypoglycemia in response to fasting [9]. Very small adrenals have been observed at autopsy [1, 3]. Histologic examination of adrenals has revealed extensive cortical atrophy with nodules of ballooned cells that stained for lipid with Oil Red O. Electron microscopy showed lamellar, needle-like lipid inclusions. It was this similarity to X-linked ALD that led to the naming of this disease. This adrenal pathology is also seen in Zellweger syndrome [37]. ACTH was demonstrated immunohistochemically in the pituitary, indicating the adrenal changes to be primary [1].

The neuropathology is characterized in some patients by polymicrogyria, as well as patchy demyelination throughout the cerebral white matter [1]. Some have had only mild abnormalities in neuronal migration and heterotopias [11]; in others the cortex and neurons appeared normal [6, 38]. The olivary nuclei were normal in all. Cytoplasmic inclusions were seen, as in the adrenal, and were like those seen in X-linked ALD, as was perivascular accumulation of lymphocytes. Demyelination of a widespread sudanophilic leukodystrophy tends to be more extensive than in X-linked ALD. It includes the cerebellum and brainstem. Periventricular rarefactions and microcalcifications have been observed [3]. Abnormalities of the gray matter include neuronal loss and inclusions in cortical neurons. Ocular histopathology includes ganglion cell loss and retinitis pigmentosa-like changes [22].

Retinitis pigmentosa may be seen in a variety of the peroxisomal disorders. Figure 63.12 illustrates the retina of a patient with defective activity of the bifunctional enzyme protein, which is a single enzyme defect in the pathway of peroxisomal β-oxidation (see Figure 63.1) rather than a defect in peroxisome assembly. Retinitis pigmentosa and, sensorineural hearing loss have also been found in PD1B deficiency [39].

Extensive hepatic fibrosis was reported in two patients at autopsy [3] and periportal fibrosis was observed at biopsy at three months of age [9]. Periodic acid-Schiff (PAS)-positive macrophages have been reported in the liver [6], but not uniformly [3]. Hepatic peroxisomes may be absent or diminished in number [12, 40].

Chemical analysis of the lipid of the brain revealed an increase in cholesterol esters and a diminution in constituents of myelin [1]. Hexacosanoic (C26:0) acid accounted for 25 percent of the total fatty acid [24, 41]. Examination of the VLCFA of the plasma and cultured fibroblasts also reveals accumulation of VLCFA. Levels are similar to those found in X-linked ALD [4]. The mean C26:C22 ratio in fibroblasts in two patients [3] was 0.5, while that in ALD was 0.7. The value for controls was 0.03. The accumulation tends to be less than that seen in Zellweger syndrome. In another patient, the ratio was 1.8 [9]. The levels of C26:0 in postmortem liver and adrenal were higher than those reported in ALD [3]. Accumulation of VLCFA has also been observed in retina [23]. Oxidation of lignoceric acid (C24:0) in cultured fibroblasts is impaired [3], and the level of activity is similar to that of cells derived from patients with ALD. Defective plasmalogen synthesis tends to be less than that of Zellweger syndrome. A systematic approach to the biochemical diagnosis of peroxisomal disorders has been set out [42]. Biochemical

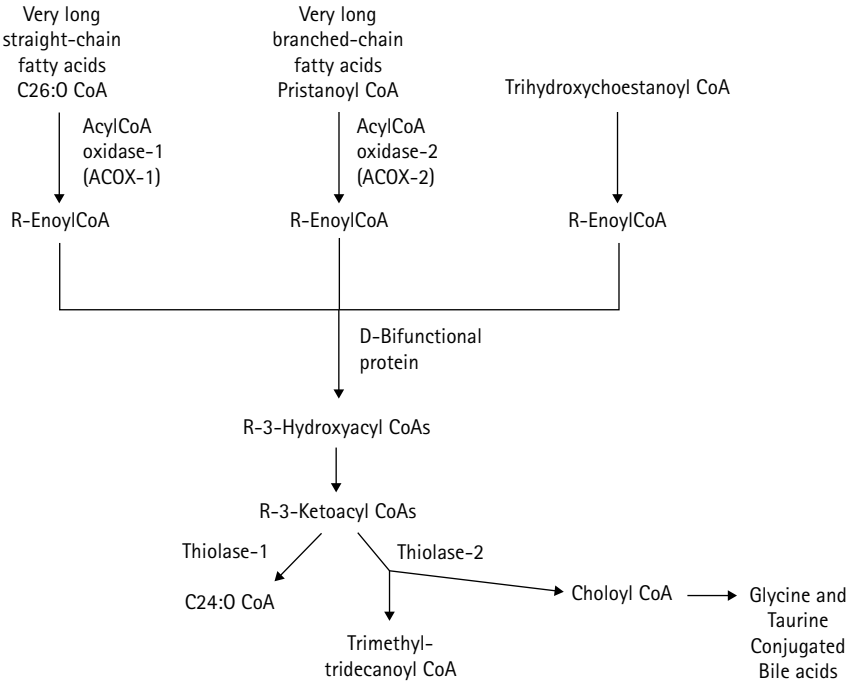

Figure 63.13 Peroxisomal β-oxidation of VLCFA.

tests are supplemented with functional studies in cultured fibroblasts, and by molecular analysis. It is clear that peroxisomal fission disorders may be elucidated in patients with normal levels of peroxisomal metabolites. Complementation studies may be used to determine which of many PEX genes is abnormal.

GENETICS AND PATHOGENESIS

All of the disorders of peroxisomal biogenesis are transmitted in an autosomal recessive fashion. Patients of both sexes have been reported with identical phenotypes. In Zellweger syndrome, consanguinity was observed in 17 of 78 patients [43]. The frequency of Zellweger syndrome was estimated to be one in 100,000 [44] and that of all disorders of peroxisome biogenesis to be one in 25,000 to 50,000 [27].

Testing of parents for levels of VLCFA in plasma and in fibroblasts yielded normal levels [3]. This is in contrast to the findings in X-linked ALD. Normal levels of VLCFA have also been found in plasma and fibroblasts of parents of patients with Zellweger syndrome [41].

Prenatal diagnosis has been accomplished in Zellweger syndrome [41], as it has been in X-linked ALD [45], by assay of cultured amniocytes, or chorionic villus samples, for VLCFA and/or the activity of dihydroxyacetonephosphate acyltransferase [46–48], as well as by demonstration that catalase activity is present in the cytosol [49]. The same approaches should also be effective in neonatal ALD. False negatives have been observed in testing chorionic villus samples, so it has been recommended that tests be followed up by testing cultured chorionic villus cells, though this would not obviate the problem of overgrowth of maternal cells [45, 50]. In patients shown to have mutations in any of the PEX genes [16, 17, 51], these mutations can be the basis of prenatal diagnosis and heterozygote detection. Prenatal diagnosis has been recommended for X-linked ALD based on testing for C26:0 lysophosphatidylcholine in dried blood spots by LC-MS/MS, and it should work for Zellweger syndrome disorders [13].

The fundamental defect in the disorders of peroxisomal biogenesis is a failure in the process of protein import into the peroxisomal matrix. This may lead to an absence of demonstrable peroxisomes as reported in Zellweger syndrome [52]. Actually, fibroblasts of these patients have been shown to have peroxisomal ghosts, or empty peroxisomal structures containing membrane proteins, but no catalase or other matrix proteins [53–56]. In milder examples, including some patients with neonatal ALD, there may be small amounts of catalase within the ghosts [57].

Peroxisomal biogenesis requires the synthesis of proteins on cytosolic polyribosomes and post-translational import to pre-existing peroxisomes, which enlarge until they divide and form new peroxisomes. Matrix proteins include catalase, the bifunctional hydratase-dehydrogenase enzyme, the thiolase, and acylCoA oxidase [6]. Peroxisomal matrix proteins carrying either a carboxy terminal peroxisomal targeting sequence (PTS1) or a cleavable amino terminal sequence (PTS2) are translocated across the peroxisomal membrane [58–60]. A defect in a peroxin, caused by mutation in a PEX gene leads to failure of protein import via either the PTS1 or PTS2 import pathway and, as a consequence, to functional deficiency of the peroxisomes.

The PEX1 gene codes for a member of the AAA protein family of ATPases, which interacts with another ATPase coded for by PEX6, and this interaction is required for matrix protein import [61, 62]. The cDNA codes for a

hydrophylic protein of 1283 amino acids [62]. Defects in the PEX1 gene account for over half of patients with defects of peroxisomal biogenesis [63, 64]. Some 90 different mutations in PEX1 have been described [65]. Two mutations, G843D [21] and 2097insT [66], are common, G843D is the most common, and the clinical effect is relatively mild [25]. PEX1 mutations lead to severe defects in matrix protein import and destabilization of PEX5, the receptor for the type 1 peroxisomal targeting signal [23]. Genotype tends to correlate with phenotype in the sense that missense mutations have been found in milder presentations and nonsense mutations, deletions, and insertions in severe disease [64]. Thus, the type of mutation can be helpful in prognosis. The G843D mutation not only leads to milder disease in the homozygote, but also appears to ameliorate the effects of genes that usually cause severe disease. It was found in homozygous fashion in at least one patient with neonatal ALD [23] and several with infantile Refsum disease. The mutation was found on one of two alleles in patients with Zellweger syndrome, as well as these two diseases [62]. A frameshift mutation in exon 18 was relatively common in Australasian patients [63]. In an assembly of 168 patients, p.G843D and c.9097 insT accounted for more than 80 percent of abnormal alleles. Class I mutations led to residual protein concentrations and function, and milder disease, while class II led to no or almost no PEX protein and a severe phenotype [67]. Mutations causing premature termination were widely distributed through the gene, while missense mutations were concentrated in the essential AAA domains of the PEX1 protein [68].

PEX2 is defective in patients in complementation group 10 [16]. The gene is on chromosome 8q21.1 [69]. It codes for a 35-kDa peroxisomal membrane protein that restores proper assembly in a CHO cell mutant that is defective in peroxisome assembly [70]. In the initial homozygous patient, a point mutation led to a premature termination of the protein, and addition of wild-type protein to cultured cells of the patient restored peroxisomal assembly. Other point mutations in this protein have been identified [71], a G-to-A 737 change that led to a cysteine-to-tyrosine change in the region of the carboxyl terminus resembling the zinc finger motif of DNA binding proteins, and a C-to-T 370 change which formed a stop codon.

The identification of the molecular defect in the PEX5 gene, defective in complementation group 2 was the result of imaginative studies [18] on the patient [9] shown in Figure 63.2. She was found to have a T-to-G transversion of nucleotide 1467 which changed an asparagine to a lysine. The same mutation was found in another, unrelated Arabian patient with a neonatal ALD phenotype in complement group 2 [72]. Another member of complement group 2 was found to be homozygous for a nonsense mutation T1168C that changed an arginine to a premature termination in PXR1 [18]. PEX1 codes for a receptor for proteins with PTS1 targeting signals, the targeting signal on the majority of matrix proteins. Fibroblasts of these patients were unable to import PTS1-containing proteins into peroxisomes. Solution of the crystal structure of the PEX5 protein revealed major changes from an open structure to a closed circle when cargo is bound [73]. Mutations within the loop led to defective import of cargo into peroxisome.

The *PEX10* gene codes for a protein of 326 amino acids and a transmembrane pattern. Mutations such as nonsense, frameshift, or splice site that removed large segments of the coding region were found in Zellweger syndrome [74], while in a mildly affected patient with neonatal ALD, a missense mutation p.H290Q was in the C-terminal Zn binding domain. The loss of *PEX10* as seen in complementation group 7 predominantly affected the input of matrix protein.

Mutation in PEX13 is responsible for patients in complementation group 13 [75]. The gene maps to chromosome 2p15. PEX13 functions as a docking factor for the cytoplasmic PTS1 receptor [75]. Newly synthesized peroxisomal matrix proteins are distinguished by the presence of PTS. Type 1 PTS has a C-terminal tripeptide ser-lys-leu, which is used for virtually all proteins destined for the peroxisomal lumen. In a patient with Zellweger syndrome, homozygosity for the nonsense mutation p.trp234ter led to loss of the transmembrane domain of the PEX13 protein [75]. In a patient with neonatal ALD with a temperature-sensitive phenotype in fibroblasts, there was homozygosity for a missense mutation p.I326T [76].

Mutations in other genes involved in the import of peroxisomal matrix proteins have been identified, in the *PEX6* [61], *PEX7* [77], and *PEX12* [78]. In addition, mutations have been observed in *PEX3* [79], *PEX16* [80], and *PEX19* [81], which code for proteins of peroxisomal membrane synthesis, all of them in patients with the Zellweger phenotype.

Mutations have been found in *PEX26*, the cause of peroxisome biogenesis complementation group 8 [82, 83]. The gene was mapped to chromosome 22q11.2. It interacts directly with *PEX6*, and through it to *PEX1*. This relatively common group contains patients with all three phenotypes. Cells of these patients have defective import of catalase and PTS2 proteins, such as 3-ketoacylCoA thiolase, but not PTS1 proteins, and the import defect is temperature sensitive [82]. The temperature-sensitive variants tend to cause less severe disease, infantile ALD, or Refsum, than the nonsensitive variants. Among 18 probands in complementation group 8, p.R98W accounted for 14 (39 percent) of variant genes, and they hypothesized a founder effect [84].

In the presence of defective processing of peroxisomal matrix proteins, these enzymes are found in the cytosol, where some, such as the oxidase and thiolase, are degraded rapidly, while catalase accumulates and is degraded more slowly than in normal cells [85]. Among the consequences of defective peroxisomal assembly is a variety of abnormalities of morphogenesis. These are most notable externally in Zellweger syndrome, but abnormal neuronal migration takes place in neonatal ALD, as well as Zellweger syndrome [85, 86]. Abnormal migration is demonstrable in fetal tissue. Neurons normally found in the outer layers

of the cerebral cortex are found in inner layers and in the white matter. Abnormal migration leads to microgyria and to thick pachygyria. Abnormal migration is not seen in rhizomelic chondrodysplasia punctata, and other peroxisomal disorders in which plasmalogen synthesis is defective, so this could not be the mechanism of the abnormal migration. On the other hand, VLCFA do not accumulate in the chondrodysplasias, so this could be involved in the abnormal neuronal pathogenesis [86]. Deficiency of plasmalogens makes cells sensitive to ultraviolet irradiation [87].

The subcellular localization of catalase correlates with the status of peroxisomes in histologic studies of tissues. In Zellweger patients, catalase is essentially all cytosolic, while in normal individuals as much as 65 percent of catalase sediments with the peroxisomal particles.

Defective peroxisomal function is manifest in pathways of plasmalogen synthesis, pipecolic acid and phytanic acid metabolism, branched chain fatty acid oxidation and cholesterol metabolism. Plasma levels of VLCFA and bile acid intermediates are elevated. The VLCFA accumulate in this condition and in Zellweger syndrome because of failure to catabolize them [88]. All of the enzymes of peroxisomal β-oxidation are defective. These enzymes are synthesized normally, but they are degraded rapidly because they cannot target into peroxisomes. Cultured fibroblasts of a patient with neonatal ALD have been shown to make mRNA normally for an enzyme of fatty acid oxidation whose activity could not be found in autopsied liver [89].

Patients with Zellweger syndrome and neonatal ALD have dicarboxylic aciduria of predominantly medium-chain length [90], such as adipic (C6), suberic (C8), and sebacic (C10) acids. This reflects the failure of peroxisomal β-oxidation. The dicarboxylic aciduria may be modest compared with that seen in abnormalities of mitochondrial β-oxidation.

Levels of docosahexanoic acid (DHA) are low in the brain, retina, liver, and plasma in patients with disorders of peroxisomal biogenesis [91, 92]. The mechanism is not yet clear, but may be a consequence of defective β-oxidation. DHA is important for the integrity of both brain and retina [93] and so may play a role in the pathogenesis of some clinical manifestations.

Bile acids are also metabolized to deoxycholic acid in peroxisomes, and precursors such as trihydroxycholestanoic acid (THCA) and dihydroxycholestanoic acid are present in high concentrations [94]. This could relate to the pathogenesis of hepatic abnormality, and levels of transaminases and bilirubin in plasma are regularly elevated in Zellweger patients.

The accumulation of pipecolic acid and its increased excretion in urine [44] is as the L-isomer. It appears to result from a failure to metabolize pipecolic acid to α-aminoadipic acid which normally takes place in peroxisomes [95].

TREATMENT

No effective treatment has been developed for the disorders of peroxisome biogenesis. The dietary regimens under exploitation in X-linked ALD were explored in the milder examples of disorders of peroxisomal biogenesis. Improvement in a patient with neonatal ALD has been reported [97] following treatment with DHA (250 mg/day), but these observations have not generally been accepted. Clofibrate has been used without success to induce the formation of hepatic peroxisomes in Zellweger syndrome. Symptomatic therapy, such as the use of anticonvulsants, may be helpful in management. Oxalate accumulates in plasma and urine, and this yields renal calculi of calcium oxalate [97]. Oral citrate maybe used to prevent calculi. A high intake of fluid is recommended. Hearing aids and spectacles may be useful.

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PART 8

DISORDERS OF PURINE AND PYRIMIDINE METABOLISM

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Introduction to the disorders of purine and pyrimidine metabolism

INTRODUCTION

Methodology, such as high-pressure liquid chromatography tandem mass spectrometry, has facilitated the diagnosis and the differential diagnosis of disorders of pyrimidine metabolism. Inherited disorders remain rare. In a study of 450 children with nonspecific neurologic manifestations, such as seizures and delayed development, two were found to excrete elevated amounts of uracil and thymine and were diagnosed as having dihydropyrimidine dehydrogenase deficiency [1].

CLINICAL ABNORMALITIES

Two patients who excreted large amounts of uracil, dihydrouracil, and β-ureidopropionate were found to have ornithine transcarbamylase deficiency (Chapter 26). In nine patients treated with Vigabatrine, β-alanine was markedly elevated. This drug inhibits γ-aminobutyrate (GABA) transaminase and so interferes with the catabolism of β-alanine.

TREATMENT

Dihydropyrimidine dehydrogenase is involved in the catabolism of 5 fluorouracil, and so patients receiving cancer chemotherapy are at risk for severe toxicity. We recommend screening for uracil-thyminuria in candidates for chemotherapy. A list of pyrimidine and other medications that might interfere with pyrimidine degradation is shown in Table 64.1.

Table 64.1 Drugs that may cause side-effects in patients with disorders of pyrimidine degradation

Compound	Therapeutic Use
Capccitabinc (5-FU prodrug)	Cancer chemotherapy
Cidovudine, cytosine	Antiviral therapy
Cytarabine	Cancer chemotherapy
Cytidine 5-monophosphate	Treatment of myopathy
Flucytosine	Antifungal therapy
Fluorouracil	Cancer chemotherapy
Gemcitabine	Cancer chemotherapy
Idoxuridine	Antiviral therapy
Lamivudine	Treatment of HIV
Oritic acid	Hepatic disease, osteoporosis
Stavudine	Treatment of HIV
Trifluridine, trifluorothymidine	Antiviral therapy
Uridine monophosphate	Use in myopathy
Vigabatrine	Anticonvulsant
Zalcitabine	Treatment of HIV
Zidovudine	Treatment of HIV

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65

Lesch–Nyhan disease and variants

MAJOR PHENOTYPIC EXPRESSION

Impaired motor development, spastic cerebral palsy, involuntary movements; self-injurious behavior; hyperuricemia, uricosuria, urinary tract calculi, nephropathy, tophi, gouty arthritis; and deficient activity of hypoxanthine guanine phosphoribosyl transferase (HPRT). In variants, hyperuricemia, gout, or renal calculi; in a neurologic variant, the phenotype is identical to that of Lesch–Nyhan disease, but self-mutilation is absent and intelligence may be normal; in another variant, the expression is of dystonia mimicking spastic diplegia and mildly impaired mental development. Variant HPRT enzymes may have activity that is 0 or as much as 50 percent of normal in hemolysates, but over 1.4 percent of control in the intact cell assay.

INTRODUCTION

Lesch–Nyhan disease was first described in 1964 [1] as a syndrome in which disordered purine metabolism, as exemplified by hyperuricemia, uric aciduria, increased turnover of an enlarged uric acid pool, and enormous overproduction of purine *de novo* was associated with a neurologic picture of athetoid cerebral palsy and bizarre, compulsive, self-mutilative behavior. The overproduction of purine from an intravenous (IV) glycine precursor was 20 times the normal value [2], whereas in adults with gouty arthritis the largest rates observed were twice the normal value. The hallmark feature of the behavior was loss of tissue because of biting. The gene was recognized early from pedigree studies to be situated on the X chromosome [3], and transmission is usually as a fully recessive character. There have been a few affected females, most reflecting a nonrandom inactivation of the normal X chromosome. The enzyme defect in hypoxanthine guanine phosphoribosyl transferase (HPRT) (Figure 65.1) was discovered in 1967 by Seegmiller and colleagues [4]. The gene was cloned in 1982 by Jolly and colleagues [5, 6]. A large number and variety of mutations have been defined [7–10].

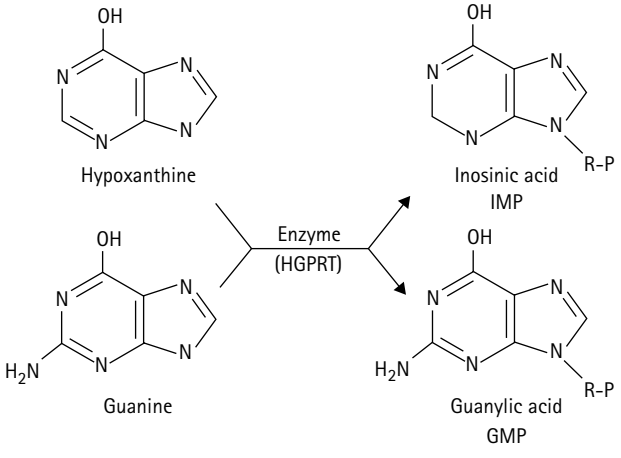

Figure 65.1 Hypoxanthine-guanine phosphoribosyl transferase (HPRT), the site of the defect in the Lesch-Nyhan disease.

CLINICAL ABNORMALITIES

Male infants with the Lesch–Nyhan disease appear normal at birth and usually develop normally for the first six to eight months. The first sign is usually the appearance of orange crystals which give the appearance of orange sand in the diapers [11], and this history is regularly obtained from the parents. This manifestation of crystalluria would permit early diagnosis, prior to the development of

neurologic or behavioral features, and I am pleased to say that we have now encountered patients who were detected early on this basis. Hematuria or urinary tract stones, as well as crystalluria, may develop during the early months of life.

Defective motor development usually becomes evident in the second six months of life. Commonly, it is the failure to reach developmental milestones or poor head control that brings the infant to attention. At this age, hypotonia is commonly evident, but some patients may have hypertonic lower extremities. The classic patient with Lesch–Nyhan disease does not learn to walk and must have some support even to sit unaided (Figure 65.2). Patients learn to sit in a chair if they are fastened securely about the chest, as well as the waist, and narrow wheelchairs are preferred (Figure 65.3). This is the preferred daytime situation for virtually all patients.

Involuntary movements have been seen in 100 percent of our patients [11]. Extrapyramidal features indicate abnormality in the function of the basal ganglia [12, 13]. Varying descriptions in the literature probably reflect varying use of the language, rather than variation in the neurologic phenotype. Increasingly, we have recognized dystonia as a major feature with its extensive contraction of agonist and antagonist muscles, dystonic posturing especially on intention, and overflow muscular contractions elsewhere. There may be flailing movements of the extremities that have been called 'ballismus'. Choreic movements are particularly common with excitement, either emotionally positive or negative. We have also observed fine, typically athetoid movements.

Opisthotonic spasms, retrocollis, or periodic arching of the back are characteristic. They begin in infancy but continue even into adult life, becoming, in some patients, a semivoluntary component of behavior. Among pyramidal features spasticity is usually considerable. Scissoring of the lower extremities is common. The increased muscle pull leads ultimately to dislocation of both hips in most patients. One of our patients had dislocated both patellae proximally. Many patients develop contractures, predominantly in flexion. Deep tendon reflexes are increased, but reflexes may be difficult to obtain because excitement engendered by the examination leads to so much activity. Babinski reflexes are regularly found, but may be absent. Spontaneous dorsiflexion of the toe (Figure 65.2) may be easier to observe than one elicited by a detailed neurologic examination. Dysfunction of ocular motor activity was reported [13] in 15 patients with severe deficiency of HPRT and the extrapyramidal manifestation of the classic Lesch–Nyhan phenotype. In contrast, in seven neurologically normal patients with lesser deficiencies of HPRT, ocular motility was normal. In the former group, ocular motor apraxia was characterized by interruption of fixation by frequent unwanted saccades toward minor visual distractions. Even the most cooperative patients could not fixate on a central target for one minute. Voluntary saccades were initiated by sudden head movement, an eye blink, or both. Immobilization of the head led to a delay in initiated voluntary saccades. Four patients had blepharospasm and two had ocular tics. These abnormalities were deemed consistent with abnormalities in the function of the basal ganglia or their connections with ocular motor centers. Seizures occur in some patients, but are not a major

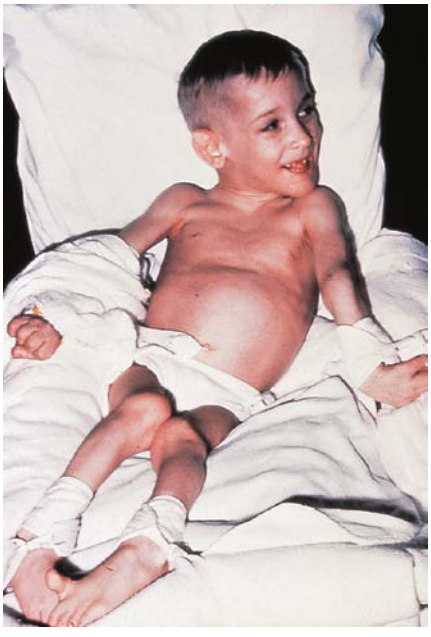

Figure 65.2 MJ: A seven-year-old boy with the Lesch–Nyhan syndrome illustrating the motor disability as exemplified by an inability to sit without support, dystonic posturing in the position of the upper extremities, spasticity, and a left spontaneous Babinski.

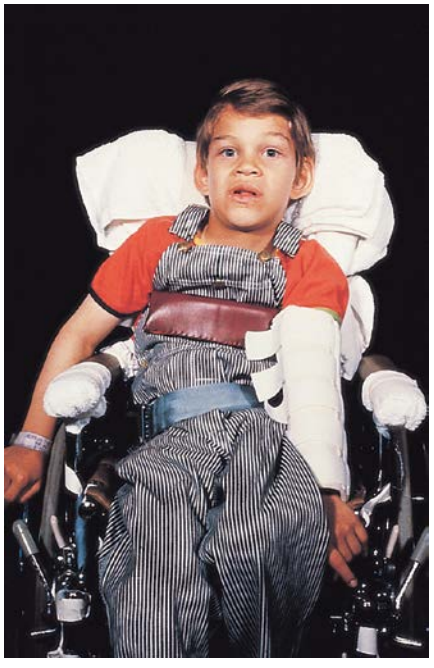

Figure 65.3 OK: A Lesch–Nyhan patient in the typical position. Supported properly, the patient can be part of the action and he can get around in a wheelchair.

feature of the disease, and the electroencephalogram is usually normal.

Impaired mental development is a prominent but controversial feature of the disease. In many patients, the IQ as tested has approximated 50. However, adequate testing is very difficult because the behavior and a short attention span get in the way of the testing. Clearly, these patients have cognitive abilities well above the level of the motor disability. A few patients have been observed in whom there has been normal or near normal intelligence. Many parents feel that their sons are intellectually normal. Two of our patients were doing grade level work in normal high schools. These patients were toilet trained. Few of the others we have studied were. All of them learn to speak, but dysarthria makes their speech difficult to understand. Despite the gravity of the cerebral disease, there is little evidence that this is a progressive, degenerative disease, but diminished neurologic function with age has been observed in adult variants (see below).

Behavioral features are integral to the disease and the behavior is compulsive and aggressive. Self-injurious behavior occurs in 100 percent of patients with the classic Lesch–Nyhan syndrome [11]. On the other hand, there are exceptions to every rule. Puig *et al.* [14] have reported two patients, one six years of age, but the other 21 years, who have not mutilated despite having uncles, and in one case a brother, who displayed the complete phenotype including self-injurious behavior. More recently, we have reported [15] a family in which a single mutation has been expressed as three different phenotypes, only one of whom had the classic Lesch–Nyhan phenotype. Two of these adults manifested deterioration of motor functions in adulthood, such that the ability to walk was lost.

The behavior is compulsive and aggressive. While aggressive activity is predominantly directed against the patients themselves, they do attempt to injure others and sometimes succeed, but the motor disability largely prevents much success. The most characteristic feature is self-destructive biting of the lips (Figures 65.4 and 65.5) and fingers (Figures 65.6– 65.8). Unlike many other patients with mental impairment who engage in self-mutilative behavior, these patients bite with a ferocity that leads to significant loss of tissue. Partial amputation of fingers has been observed (Figure 65.8).

The differential diagnosis of self-injury of this disease includes the De Lange syndrome and dysautonomia. Self-injurious behavior has also been observed in as many as 58 percent of boys with fragile X syndrome [16]. The behavior consisted predominately of biting fingers or the dorsum of the hand or hitting the head. Information on severity, for example loss of tissue or amputations, was not provided. In patients with Prader-Willi syndrome [17, 18], the behavior consisted of picking the skin over an extensive body area. In Smith-Magenis syndrome, self-injurious behavior was described [19] as near universal. Hand biting was the most common behavior in these patients too, but they also manifested some unusual behaviors, including: picking at

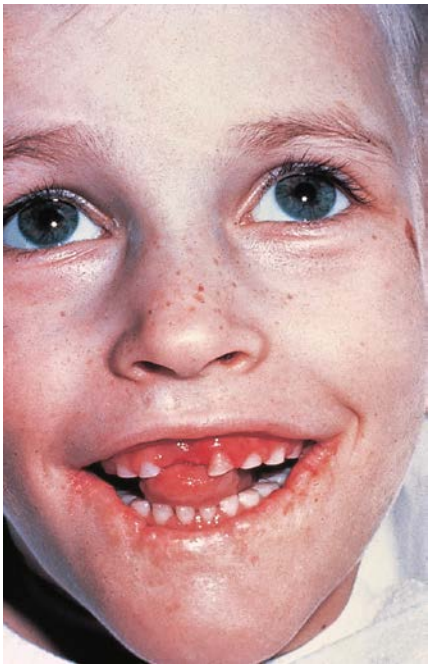

Figure 65.4 MJ: The degree of the mutilation of the lip is relatively mild.

fingers or toenails until they bled, onychotillomania, and even complete removal of the nail, and polyembolokoilamania (the inserting of foreign objects into orifices of the body). It is thought that in this disorder there may be peripheral neuropathy. The behavioral phenotype of Smith-Magenis syndrome also includes extreme disturbance of sleep and

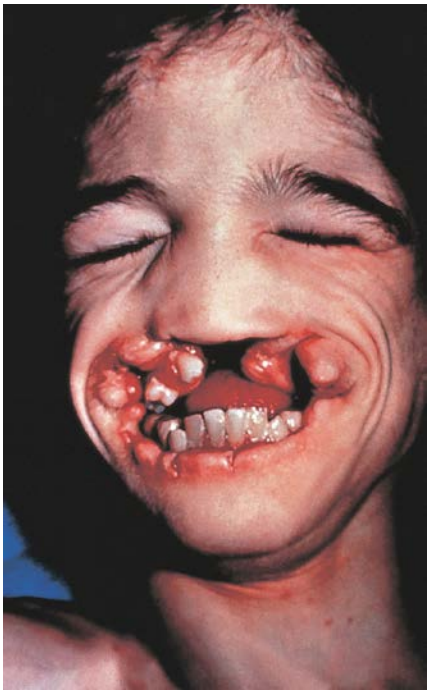

Figure 65.5 JJ: A 14-year-old boy, illustrating an extreme degree of mutilation around the face.

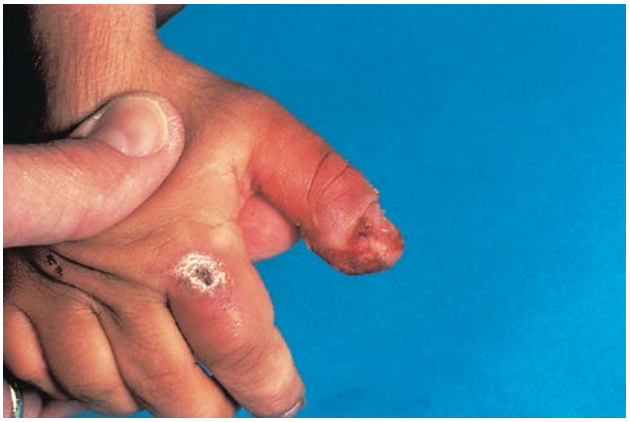

Figure 65.6 Freshly bitten lesions of the thumb and forefinger of a six-year-old Saudi boy with the Lesch–Nyhan syndrome.

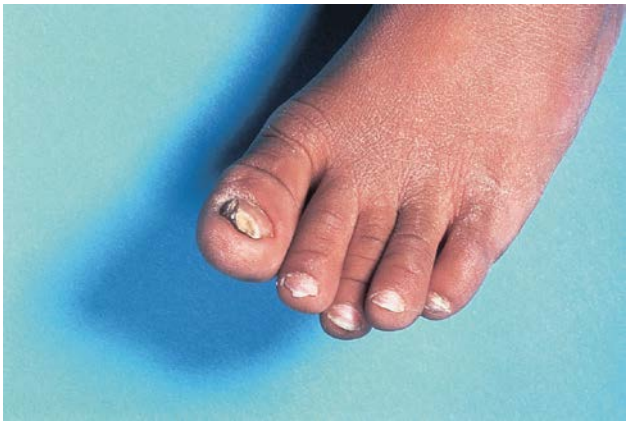

Figure 65.7 This same boy as illustrated in Figure 65.6 managed to bite his great toe as well. He also had loss of tissue from biting the lower lip, banged his head and created sores on his chin by rubbing it on the floor.

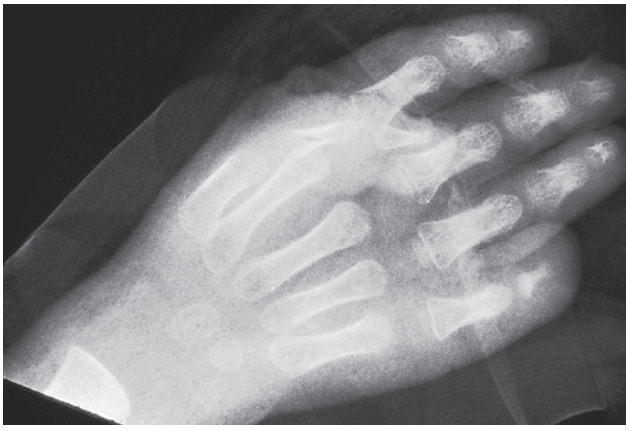

Figure 65.8 MJ: Roentgenogram of the left hand, illustrating partial amputation of the fifth finger.

a "self-hugging" stereotype. The differential diagnosis does not really include sensory neuropathy and indifference to pain. Those patients tend to look like pugilists and the injuries are accidental. Lesch–Nyhan patients do not have sensory abnormalities; they scream in pain when they bite themselves and cry in terror of its anticipation. As patients become older, they learn to become aggressive with speech. Four-letter Anglo-Saxon expressions are common. Males appear to have a considerable interest in the opposite sex. This sometimes leads to inappropriate groping; more often it leads to frustration.

Apraxic discoordination of the lips and tongue make feeding difficult and swallowing is imperfect. In addition, most have required some teeth extraction in order to protect against damage by biting. Patients with this disease feed poorly. Virtually all vomit, and this, too, seems to be incorporated into the behavior. In most patients, growth in height and weight are well below the norms for chronological age [11]. Autopsy studies have revealed no consistent abnormalities in the brain and a number of brains have been judged to be normal. So, too, have routine neuroimaging studies. However, quantitative magnetic resonance imaging (MRI) comparison of patients and age-matched controls has revealed a 34 percent decrease in the volume of the caudate [20], which appeared to reflect abnormal development as opposed to atrophy.

Hyperuricemia is present in virtually all patients. The concentration of uric acid in the plasma is usually between 9 and 12 mg/dL, which level is at the limit of solubility of urate in plasma. Patients with some degree of acute or chronic glomerular insufficiency may have higher concentrations of uric acid, and some who are very efficient at excreting urate may have lower values, occasionally in the normal range. The clinician must be careful, however, at accepting a conclusion that a plasma uric acid is normal from a laboratory whose norms were established on adult males in whom hyperuricemia is common. A report [21] concluded that a one-year-old had a normal plasma concentration of uric acid when the level was 7.6 mg/dl.

We have reported that after the first 80 hours of life the normal uric acid in plasma is less than 3.4 mg/d [22]. All patients excrete large amounts of uric acid in the urine. Twenty-four-hour excretions of 600–1000 mg are the rule in patients weighing 15 kg or more. Throughout childhood, patients with this disease excrete three to four times as much uric acid as do control individuals of comparable size. In relation to body weight, they excrete 40–70 mg of uric acid per kg. Another pitfall in interpreting uric acid data arises from the propensity of microorganisms to consume purines including uric acid; conditions of collection of a 24-hour sample at room temperature are ideal for bacterial purposes. For this reason, it is best to avoid collecting 24-hour samples, except for research purposes under which each sample is added to the batch in the freezer as soon as obtained. For diagnostic purposes, it is more convenient to collect a fresh sample and analyze promptly for uric acid and creatinine [23]. These patients regularly excrete 3–4 mg

of uric acid/mg of creatinine, while in control individuals older than one year of age the level is less than 1.

The clinical consequences of the accumulation of large amounts of uric acid in body fluids are manifestations classic for gout. These patients pass large quantities of urate crystals in the urine (Figure 65.9). Episodes of hematuria and crystalluria are the rule and may cause abdominal pain. Urinary tract calculi are regularly observed (Figures 65.10 and 65.11) and they may occur as early as the first months of life; they lead regularly to urinary tract infections. In the absence of treatment, urate nephropathy develops as a result of the deposition of sodium urate in the renal parenchyma. Death from renal failure at less than ten years of age was the expected outcome before the development of allopurinol. Tophi may be seen in those unusual patients who survive without treatment beyond ten years (Figure 65.12). Acute gouty arthritis is even more rare, but has occurred uniformly in untreated patients reaching adult life. Chronic tophaceous gout has been observed (Figure 65.13) [24].

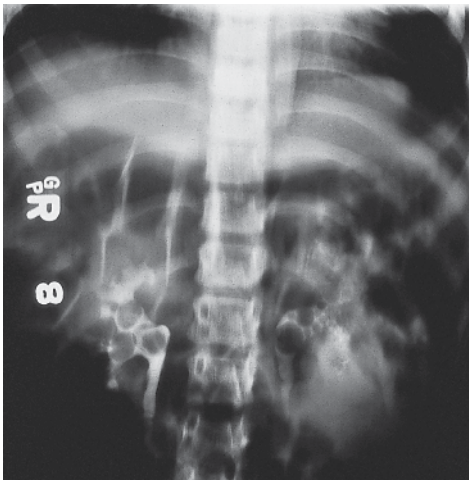

Figure 65.11 IV pyelogram of a patient with the Lesch–Nyhan disease. There were numerous radiolucent calculi.

Figure 65.9 Uric acid crystals in the sediment from a fresh, centrifuged sample of urine viewed through polarized light. (Reprinted with permission from Stapleton FB and Linshaw MA. *N Engl J Med* 1994; 330: 762.)

Figure 65.12 A 17-year-old boy with prominent tophaceous deposits in the ears. The violaceous inflammatory reaction is unusual around tophi. It subsided following treatment with colchicine.

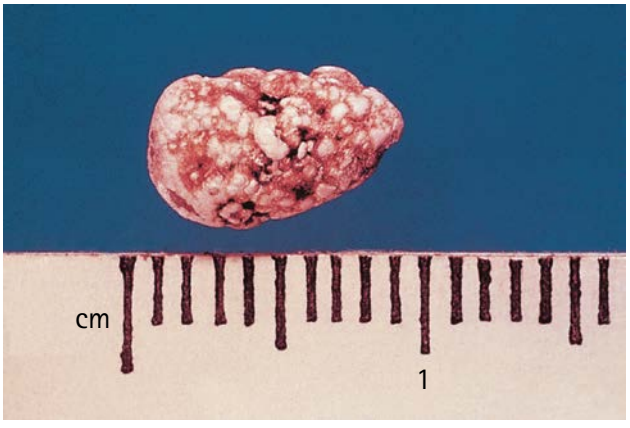

Figure 65.10 Urinary calculus recovered from the urine of a patient with HPRT deficiency.

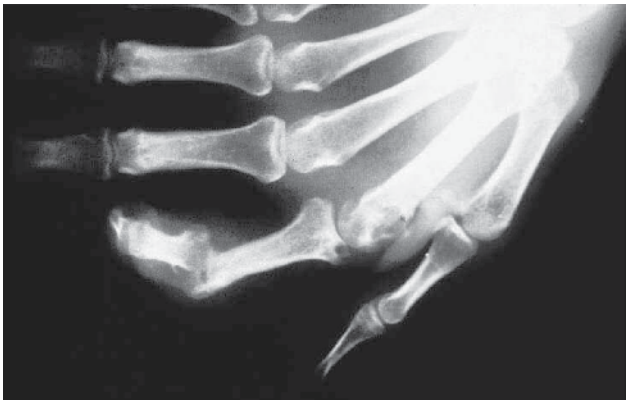

Figure 65.13 Roentgenogram of an adult patient with HPRT deficiency illustrating typical lesions of chronic tophaceous gout.

Quantitative studies of brain volume in patients with Lesch–Nyhan disease, variants and control [25] have yielded significant reductions in volume of the caudate and putamen, as well as their downstream targets the thalamus cerebral cortex and limbic regions. Parietal occipital and cerebellar regions were completely spared. These observations were interpreted to indicate a developmental, other than a degenerative abnormality.

Sudden unexplained death has been relatively common in older patients with classic Lesch–Nyhan disease. We and others have speculated that forced retrocollis and cervical cord injury might be responsible.

The discovery of the enzyme defect in Lesch–Nyhan disease was followed shortly by the recognition of deficiency of the enzyme in patients with gout or urinary tract calculi [26]. Initial expectations that these populations might be quite large have turned out not to be true, and most patients with abnormal HPRT enzymes have classic Lesch–Nyhan disease. Nevertheless, a certain number of variants have been described – enough that assay for HPRT activity should be performed in any patient with overproduction hyperuricemia, and any hyperuricemic patient with a diagnosis of cerebral palsy; especially if the prenatal and perinatal course were normal. In an infant found to have HPRT deficiency, the distinction between the classic Lesch–Nyhan prognosis and that of the variant forms is of major importance.

The populations of variant patients initially described [26, 27] had hyperuricemia, with gout or renal stones and HPRT enzyme activity greater than zero. For this reason, they have been referred to as partial variants. However, additional experience shows that some of these patients have zero activity in the erythrocyte lysate assay and cannot, in this way, be distinguished from patients with Lesch–Nyhan disease. The phenotype of the patient with the classic partial variant enzyme consists of manifestations that can be directly related to the accumulation of uric acid in body fluids. The central nervous system and behavior have been described as normal. However, recent observations suggest that this may be simplistic [27]. The earliest presentation, as in the Lesch–Nyhan disease, should be orange sand in the diaper. Advantages of the earliest diagnosis possible should be effective therapy to prevent renal complications. Renal calculi have been observed even early in childhood [26]. Such patients may present with hematuria, colic, urinary tract infection, or passage of a stone (Figures 65.10 and 65.11). There may be acute obstruction of one or both ureters and hydronephrosis. Crystalluria is so massive that an intercurrent infection that leads to vomiting or dehydration may result in complete obstruction of the ureters with sludge requiring emergency surgery and ureteral lavage. We have observed this complication in which the crystals were of uric acid, but also of oxypurinol [28], the oxidation product of allopurinol. In the initial evaluation of follow up of a patient with overproduction hyperuricemia, renal ultrasound or an IV pyelogram is essential to assess for the presence of uric acid or xanthine stones, which are radiolucent. A radiopaque stone in a patient with hyperuricemia indicates the codeposition of calcium salts. Ultrasonography (Figure 65.14) may reveal echogenic crystals in the substance of the kidney. Untreated accumulation of uric acid can lead to urate nephropathy and renal failure.

Another presentation is with painless hematuria in a pattern that suggests a diagnosis of hemorrhagic cystitis or glomerulonephritis (Figure 65.15) [29]. Cystourethography

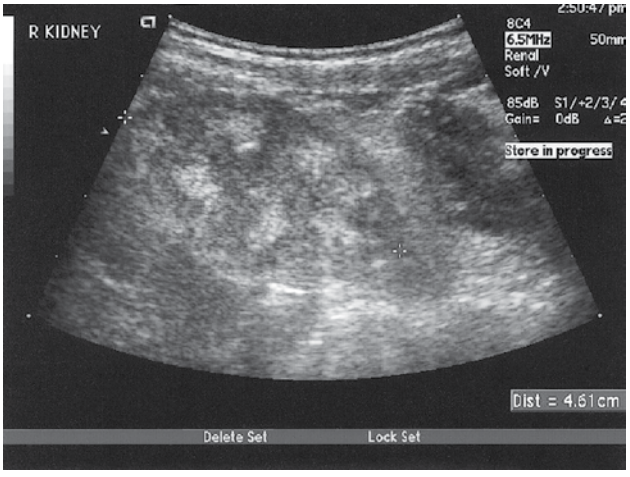

Figure 65.14 Ultrasonograph of the kidneys of an infant with HPRT deficiency. Uric acid crystals are echogenic.

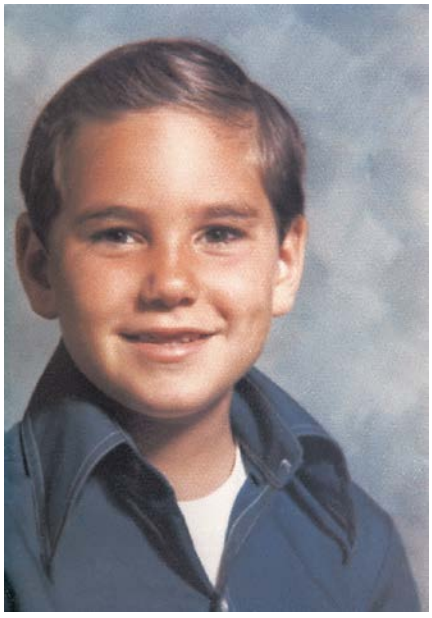

Figure 65.15 TL: A seven-year-old boy with a variant enzyme HPRT whose initial presentation was with painless hematuria. The initial episode was thought to be hemorrhagic cystitis, but recurrent episodes suggested chronic glomerulonephritis. Biopsy was normal. HPRT activity was 3 percent in the erythrocyte assay at high concentration of substrates.

in this patient led to transient hypertension, oliguria, and azotemia; following anesthesia and renal biopsy, oliguria/anuria recurred, and the BUN (blood, urea, nitrogen) rose to 80 mg/dL.

A majority of reported patients with partial variants have presented with gout [30]. Acute attacks of gouty arthritis and tophi usually occur first in adult life even though the hyperuricemia has been present since birth. It appears to require approximately 20 years of hyperuricemia before the conditions are appropriate for precipitation of the needle-like crystals of urate that produce the inflammatory response of the acute attack of arthritis. There are exceptions to every rule and we have found a variant enzyme in a patient in whom acute attacks of arthritis began at one year.

Behavior is normal in these variant patients (Figure 65.16). Antisocial behavior that has rarely been described may be completely unrelated to the defect in HPRT [31].

A quite different phenotype is what we have called the neurologic variant [32]. This picture, which has been observed in a small but important group of patients, is characterized by a neurologic examination that is identical to that of the classic Lesch–Nyhan patient. These patients are generally diagnosed as having cerebral palsy or athetoid cerebral palsy. They are confined to wheelchairs and unable to walk. The index patient was reported by Catel and Schmidt [32], as a patient with the Lesch–Nyhan syndrome in whom intelligence and behavior were normal. He has since been followed by Manzke and colleagues [33], and we had the opportunity to study him just after his graduation from university (Figure 65.17). He spoke English and German. There were no abnormalities of intelligence or behavior. His variant HPRT was zero in erythrocyte lysates, but activity was readily distinguished from Lesch–Nyhan disease by the intact cell assay [34]. Activity approached 10 percent of control, and there was enough activity to permit kinetic studies [35]. A maternal uncle had had a similar syndrome. Other patients have since been studied in whom variant enzymes have produced this phenotype [36, 37]. Behavior is normal and intelligence is normal or nearly normal.

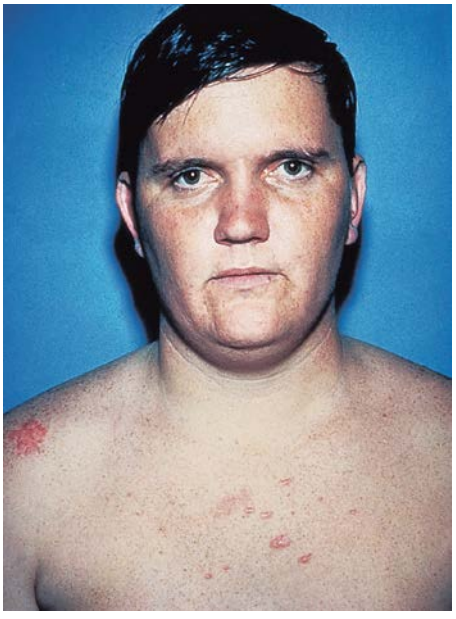

Figure 65.16 RL: A young man with a partial variant HPRT, was diagnosed after he passed a uric acid calculus during his second episode of renal colic. He and three male relatives displayed approximately 5 percent of the control level of HPRT by erythrocyte assay.

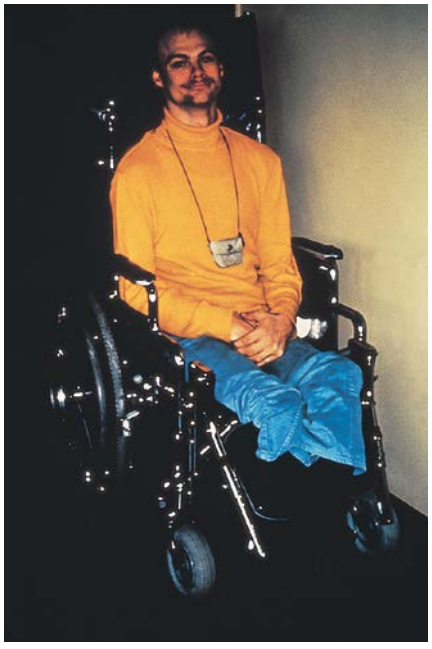

Figure 65.17 HCB: A 21-year-old patient with the classic neurologic variant [34]. Neurologic manifestations were identical to those of the Lesch–Nyhan disease, but intelligence and behavior were normal. A variant enzyme was documented by the intact cell assay.

Virtually complete deficiency of HPRT in erythrocyte lysates was recently reported [38] in a 28-year-old patient with hyperuricemia and gout, along with deficiency of glucose-6-phosphate dehydrogenase, who had mild developmental impairment, mild dysarthria, and hypotonia. Puig and colleagues [14] observed a variant patient who was intellectually normal, but had such spasticity and was so dystonic that he could not walk.

Another phenotype has been observed in a family with an HPRT variant that we have called $HPRT_{Salamanca}$ [39]. In this pedigree, four males in three generations had an identical phenotype, the most prominent feature of which was referred to as spastic diplegia. They were all able to walk, albeit with a spastic gait. The boy we studied (Figures 65.18 and 65.19) wore out the tops of the toes of his shoes because of the way he dragged his feet. Muscle tone and deep tendon reflexes were much more increased in the legs than in the arms. Babinski responses were positive bilaterally. There was a bilateral pes cavus and exaggerated lumbar lordosis. Mental impairment was mild. Involved members of the family were effectively employed as migrant grape

Figure 65.18 AA: A boy with $HPRT_{Salamanca}$. He had a pronounced spastic diplegia and a mild degree of developmental impairment.

Figure 65.19 AA: Rear view walking illustrating the dragging of his toes.

pickers in Southern France. The proband had developed tophaceous gout by 32 years of age. Each of the involved members of the family had clinodactyly of the fifth fingers and proximally placed thumbs. The abnormal enzyme displayed approximately 8 percent of control activity in the whole cell assay. Other abnormalities observed in Lesch–Nyhan patients, such as megaloblastic anemia or testicular dysfunction, may be seen in variants. Many variant individuals have shown a considerable interest in the opposite sex. Some have married.

We made a distinction early on as to two and then three different phenotypes of the HPRT deficiency. Now it is clear that there are at least four. Neurologic variants have been usefully distinguished between those who can and those who cannot walk [14]. With increasing experience, the distinctions begin to seem artificial and there is now at least one mutation reported in which three different phenotypes were seen in a single family [15]. The main argument for the distinction is the issue of prognosis in an infant newly diagnosed as having HPRT deficiency. This is a major issue for parents.

Variations in phenotype in patients with the same mutation have rarely been encountered. In one family [40], two young cousins had very mild developmental delay; cognitive testing put them in the low average range. Their grandfather was severely impaired cognitively, and he had been when he was their age. He also had neurologic abnormalities that interfered with motor function. His HPRT activity in intact fibroblasts was 27 pmol/110 nmol UV (control 1160–5228). Those of the cousins were 125 and 369. mRNA expression (real time RT-PCR) indicated that copy number of mRNA was very much lower than those of his grandchildren. Their mRNA expressions were consistent with their levels of enzyme activity [41].

Among patients with variant HPRT enzymes, a guanine to adenine change at nucleotide 143 has been encountered relatively commonly. This is consistent with independent mutation at a CpG motif where cytosine may be methylated and then deaminated to form thymine. This particular mutation changed arginine at amino acid 48 to histidine. Generation of a recombinant enzyme with this mutation yielded a protein with no difference from wild type in the Km for hypoxanthine or guanine. However, the mutant enzyme had markedly greater instability to heat at 37°C or 55°C [42]. Phenotypic variation was described among ten individual members of eights families with this mutation.

Ceballor-Picot I *et al.* reported a large family with a mutation (c.203T>C in the gene which led to a substitution of proline for leucine (p.Leu68Pro) [43]. Members had an attenuated phenotype in which patients could walk but with a stiff labored gait and dystonia. Behavior was generally normal, but two patients had self-injury.

GENETICS AND PATHOGENESIS

The molecular defect in the Lesch–Nyhan disease is in the activity of the enzyme HPRT (E.C.2.4.2.8.) (see Figure 65.1). This enzyme catalyzes the reaction of hypoxanthine or guanine with phosphoribosyl pyrophosphate (PRPP) to form their respective nucleotides, inosinic, and guanylic acids. The enzyme is present in all cells of the body. It is particularly active in the basal ganglia and testis. The defect

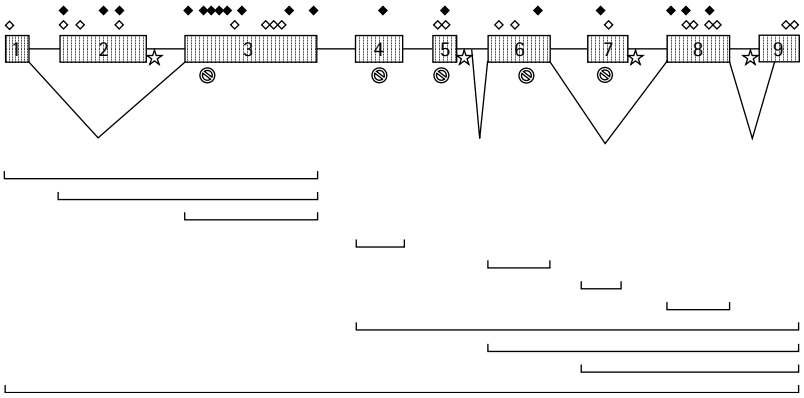

Figure 65.20 The HPRT gene. The nine exons and sites of mutations are shown. The open diamonds, variants; filled diamonds, classic Lesch–Nyhan patients; stars, frameshifts; stops signs, stop codons. Deletions of various sizes are shown below, virtually all in Lesch–Nyhan patients.

is readily detectable in erythrocyte hemolysates and in cultured fibroblasts. In the erythrocyte, quantitative assays reveal no activity in patients with classic phenotype.

HPRT is determined by a gene on the long arm of the X chromosome at Xq26-27 (Figure 65.20). The disease is transmitted as an X-linked recessive trait (Figure 65.21). It is essentially a disease of the male, occurring at a frequency of approximately one in 380,000 births. Six females have been observed [44, 45].

The heterozygous carrier can be detected by assay of the enzyme in individual cells, such that the two populations of cells specified in the Lyon hypothesis are demonstrated. This has been accomplished by cloning or pharmacologic selection in thioguanine or azaguanine. A more convenient, but still tedious, method is hair root analysis [46], which takes advantage of the largely clonal nature of individual hair follicles, but requires the plucking of at least 30 individual hairs and analysis of enzyme activity in each one. Definition of the molecular defect in an individual family permits direct testing for carriers of the mutation. In families in which the mutation is known, it is the method of choice. It can be simplified, especially where a new restriction site is created or an old one eliminated by the mutation. In informative families, linkage analysis of restriction fragment length polymorphism (RFLP) has been used for heterozygote detection [47]. Study of the carrier status of mothers has indicated that the incidence of a mutation is considerably less than the one-third of

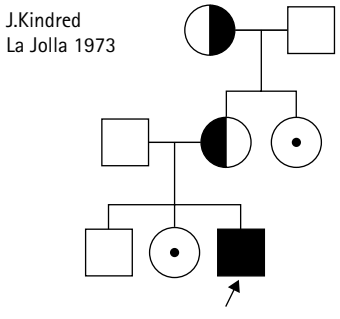

Figure 65.21 Pedigree of MJ. Symbols employed were black box (hemizygous male), half black circle (heterozygous female), and circle with a dot (a female tested and found to be normal).

affected patients predicted by population genetics theory for an X-linked lethal gene [48]. There is some evidence for an effect of paternal age and of mutation occurring in the genesis of the carrier mother. Simple testing of erythrocytes or leukocytes for enzyme activity is not useful for carrier detection, because activity in heterozygotes is virtually always normal [48], an index probably of selection against mutant cells.

Prenatal diagnosis has regularly been accomplished by assay of the enzyme in amniocytes [49–52] or chorionic villus samples, and a considerable number of affected and nonaffected fetuses has been detected. Nucleotidase activity, which is high in villus material, may lead to the breakdown of newly formed IMP and thus cause a false-positive diagnosis of an affected fetus [51] so it is important to inhibit the nucleotidase during the assay. Among normal individuals, a certain amount of RFLP has been identified and the linkage appears to be quite tight. This has permitted its use in informative families in which the mother carries identifiable alleles for prenatal diagnosis, as well as for the detection of heterozygosity [47]. In families in which the mutation is known, determination of its presence or absence is the method of choice for prenatal diagnosis.

The gene for the HPRT enzyme has been cloned and its nucleotide sequence determined [5, 6]. It spans more than 44 kb of DNA; its coding region contains 654 nucleotides in nine exons. The mRNA is 1.6 kb, but the reading frame approximates 700 bases and the enzyme contains 217 amino acids [53], which forms a tetramer.

The introduction of the polymerase chain reaction has permitted the identification of a number of mutations in patients with HPRT deficiency [7, 8, 53–60]. In the most recent summary, 302 mutations were recorded [7, 8]. In most families studied, a unique mutation has been found. The same mutation has only rarely been found in unrelated pedigrees. In most patients, the gene is not grossly altered; on the other hand, in classic Lesch–Nyhan patients, the results of the mutation have been major ones leading to essentially no activity of the enzyme. In eight classic Lesch–Nyhan phenotypes [54], an entire exon was deleted in two; and in three, nonsense mutations led to a stop mutation and hence a markedly truncated protein. In Lesch–Nyhan patients, some large deletions have been detectable by

Southern blot analysis. The number of coding sequence deletions in the latest update numbered 66 [8]. In classic Lesch–Nyhan patients, a complete spectrum of mutations has been observed including major disruptions, such as deletions, insertions, frameshift mutations leading to exon skipping and stop codons leading to truncated unstable proteins which are readily degraded [7]. Missense mutations were classically nonconservative in the classic phenotype; for instance, an aspartic acid for a glycine at position 16, a leucine for a phenylalanine at position 74, and a tyrosine for an aspartic acid at position 201. A very small number of CpG mutational hot spots were identified, for instance at arginine 51 and 170 of the HPRT protein. These are thought to represent deamination of 5-methylcytosine to thymine changing the message for arginine to stop [58]. This mutation has occurred 11 times among reported patients [7, 54]. Among other mutations of the gene, duplications have been reported, one of which resulted from recombination of Alu sequences in introns 6 and 8 [57]. This patient, though 22 years and with severely impaired mental development, had no self-injurious behavior; so he could represent a variant.

In contrast to the mutations in the classic Lesch–Nyhan disease, the majority of the variants had missense mutations. Most had a single nucleotide change. Among seven variant patients reported from Spain [14], none had mutations that predicted altered protein size. Of the 302 mutations summarized by Jinnah *et al.* [7, 8], there were two deletions in variant patients in contrast to six in Lesch–Nyhan patients. The one insertion observed in a variant added a single amino acid and did not alter the reading frame. Changes, such as a leucine to valine at codon 78, appeared relatively conservative and led to a variant phenotype whereas a leucine to glutamine change at 78 led to a Lesch–Nyhan phenotype [7]. In $HPRT_{Salamanca}$, there were two mutations: a T-to-G change at position 128 and a G-to-A at 130. These led to conversions of two adjacent amino acids at positions 43 and 44 in the protein: a methionine to an arginine and an aspartic acid to an asparagine. These alterations appear nonconservative. However, they illustrate another issue; mutations in the variants have tended to cluster in the amino end of the molecule. In contrast, Lesch–Nyhan alterations in this area tend to be those with stop codons. Mutations of HPRT that alter RNA splicing have been summarized by O'Neill and colleagues [58]. They accounted for 12.5 percent of all known mutations, those of human disease and those induced by mutagens. These are interesting because they have pleiotropic effects leading to multiple RNA products. These authors also pointed out that harmless polymorphisms have not been described in the HPRT gene.

In summary, it is clear that major interruptions of the gene and truncations of the protein lead to classic Lesch–Nyhan disease. Most missense mutations also lead to the classic phenotype, but among variants virtually all have missense mutations or frameshifts. A strong case for correlation between residual enzyme activity and, disease severity was made [59] in a study of 44 mutations expressed in vitro and purified.

Prenatal diagnosis and heterozygote detection can readily be carried out in a family in which the mutation is known. In one family [60], a nonsense mutation of the CpG site for arginine 169 was identified in a fetus and five female heterozygotes, in three of whom X chromosomal mosaicism could not be demonstrated by repeated hair root analysis or by selection of fibroblasts in azaguanine and thioguanine. Presumably, this represents an extreme example of selection against the mutant cell. We recommend prenatal diagnosis in pregnancies in which a mother has had an affected infant even if she is found not to be a heterozygote in order to avoid the problem of gonadal mosaicism. Also, as there have now been affected females documented, we recommend prenatal assessment of HPRT status even when the fetus is found to have two X chromosomes.

Establishment of the diagnosis in a patient with the typical phenotype requires determination that the activity of HPRT in a hemolysate is zero. However, some patients with different variants and different clinical phenotypes may also have zero activity in the erythrocyte assay. Assay of the enzyme in intact cultured fibroblasts has permitted the distinction of these populations: patients with Lesch–Nyhan disease have activity that is less than 1.4 percent of normal [61]; the variants have all had more activity.

Patients with variant forms of abnormal HPRT can also be characterized as different from normal by the assay [62–64] of the enzyme in erythrocytes which identifies the presence of enzyme deficiency. Families in which there is a considerable amount of activity in the erythrocyte are also distinguishable from classic Lesch–Nyhan variants. There may be 1 percent, 5 percent, 15 percent, or more of the normal level of activity; and in a kindred in which there are a number of involved members (Figure 65.20) [62], each involved member has the same deficiency. All four males in one kindred (Figures 65.16 and 65.22) had 5 percent of control activity. In other families, altered kinetics may be illustrated by different activity at saturating concentrations of substrates than at low concentrations; therefore, we routinely carry out the assay under these two conditions. In one of our patients with gout, the erythrocyte activity was 60 percent of control [62].

On the other hand, the difficulty arises because a number of patients with phenotypes very different from the Lesch–Nyhan disease have been found to have no activity of HPRT as measured in erythrocyte or fibroblast lysates. This is a likely result of the fact that structurally abnormal enzymes are often unstable, and activity disappears rapidly once cell walls are broken. It is for this reason that no correlation has been found between the level of activity of the enzyme in hemolysates and clinical features in patients [63]. This is not because patients with the Lesch–Nyhan disease display any appreciable activity in these assays; it is because patients with quite mild phenotypes, including no central nervous system abnormality, also have no activity.

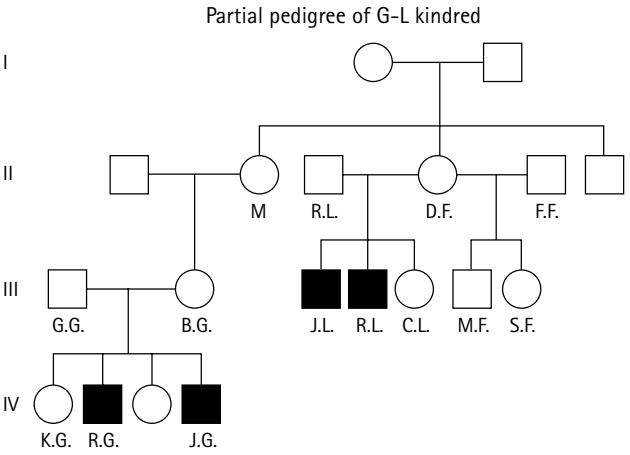

Figure 65.22 Partial pedigree of the G-L kindred (see Figure 65.16) [24].

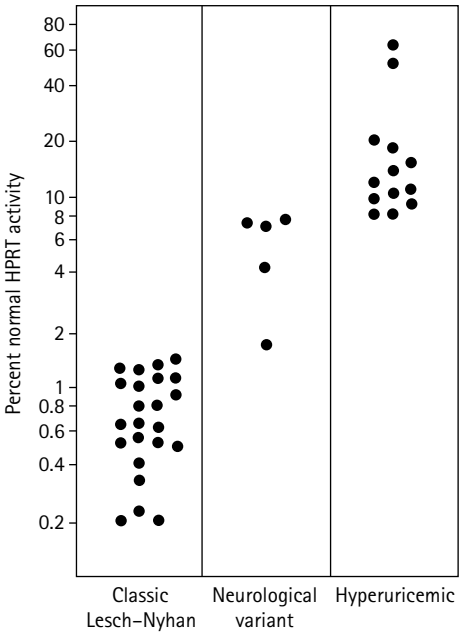

Figure 65.23 Activity of HPRT in intact fibroblasts. The level of enzyme activity was roughly inversely proportional to the degree of clinical severity. Actually, the values fell into three groups, correlated with phenotype: the Lesch–Nyhan, the neurologic variant, and the classic partial variant.

This problem was solved by the development of the more physiologic method in which enzyme activity is assessed in intact cells [64, 65]. Cultured fibroblasts are incubated with ^{14}C-hypoxanthine, the products are separated by high-performance liquid chromatography, and the total number of picomoles of isotope incorporated into purine compounds is expressed per nanomole of total purine compounds present [35]. The method permits the determination of kinetic properties of the enzyme. The Km for hypoxanthine found in normal fibroblasts was identical to that of purified human HPRT, and a number of kinetic variants have been documented [65]. The patient illustrated in Figure 65.17 was shown with this method [34] to have a variant different from the classic Lesch–Nyhan enzyme; his HPRT converted 9 percent of ^{14}C-hypoxanthine and 27 percent of ^{14}C-guanine to products that were mostly adenine and guanine nucleotides. In a series of patients with varying phenotypes, a roughly inverse correlation was obtained between enzyme activity and clinical severity (Figure 65.23) [35]. The activity obtained with hypoxanthine correlated better than did that obtained with guanine, and for this reason, we routinely carry out the assay with hypoxanthine substrate. In this analysis, Lesch–Nyhan patients have displayed activity below 1.2–1.4 percent of normal, and the classic partial variants all had greater than 10 percent of control activity. The neurologic variants have had intermediate levels of activity. Patients with $HPRT_{Salamanca}$ had 7.3 percent of control activity. Torres and Puig [66] have employed an intact erythrocyte assay with similar results. They have referred to the classic Lesch–Nyhan patients as type 4 and those with no neurologic findings as type 1. Neurologic variants type 2 and 3 were distinguished by the fact that in type 2 they could walk.

Variant enzymes have been observed to have a variety of other properties that have aided their characterization as distinct, such as unusual sensitivity to fluoride [67], unusual kinetic properties, or altered heat stability [23, 24]. Electrophoretic analysis has revealed mobilities that were both faster and slower than the normal enzyme [65]. Antibody generated against normal human HPRT has demonstrated the presence [68] and the absence of cross-reacting material (CRM). In all the variants studied, the activity of adenine phosphoribosyl transferase (APRT) is increased; the level may approximate 150 percent of control.

When the primary structure of normal human HPRT was determined [69], it became possible to determine the amino acid substitutions in variants in which a substantial amount of activity permitted purification and the preparation of peptide maps. For instance, in $HPRT_{Toronto}$, the substitution of glycine for arginine at position 50 is far removed from the binding sites for substrate, and, consistent with this, the kinetics were normal, and the phenotype was that of a classic hyperuricemic variant. In similar fashion, the transposition in $HPRT_{London}$ was far removed from substrate binding sites.

Overproduction of purines via the *de novo* pathway is probably largely a consequence of an accumulation of phosphoribosylpyrophosphate (PRPP). Increased amounts of uric acid accumulating in body fluids lead to the clinical manifestations of gout and its renal complications. It is important to remember that normals for routine chemistry laboratories in general hospital are established for adults. A serum concentration of 5 mg/dL in an infant or child is distinctly elevated [70]. The degree of overproduction in the variants is the same as in the Lesch–Nyhan disease. It is as though the production of purines has been reset at maximum or at a similar high plateau; they all have the

same elevation in the amounts of uric acid in the urine and in the blood.

In the presence of defective activity of HPRT, concentrations of PRPP rise, and there may be diminished feedback inhibition of glutamine amidotransferase by purine nucleotides. The rate of synthesis of purines via the *de novo* pathway increases markedly as studied *in vivo* with labeled glycine. Concentrations of hypoxanthine have been found to be elevated in the cerebrospinal fluid (CSF); levels were four-fold greater than those of control individuals [71]. Concentrations of uric acid are not elevated in the spinal fluid.

The pathogeneses of the cerebral and behavioral features of Lesch–Nyhan disease are not clear, while those features that are shared with patients with gout are clearly consequences of the accumulation of uric acid. Substantial evidence indicates that there is an imbalance of cerebral neurotransmitters. The best evidence for altered dopaminergic function came from the postmortem study of the brains of three patients in which there was statistically significant depression of dopaminergic function in the caudate, putamen, nucleus accumbens, and external pallidum [72].

The concentration of dopamine was decreased in the caudate nucleus of the brains of two more patients with Lesch–Nyhan disease [73]. In these brains, immunoreactive D_1 and D_2 dopamine receptors were increased in the putamen. Increase in these receptors has also been observed in positron emission tomography (PET) studies in Parkinson's disease [74]. These findings support a theory of dopamine supersensitivity in Lesch–Nyhan disease [75].

Levels of homovanillic acid (HVA) in the spinal fluid were found to be low [76]. Studies of PET with ligands specific for targets in the basal ganglia revealed reduction in binding to dopamine uptake transporters by 73 percent in the putamen and 56 percent in the caudate [77]. Another study showed over 60 percent reduction in fluorodopa uptake [78]. Further evidence for neurotransmitter imbalance was the transient cessation of self-injurious behavior following treatment with the serotonin precursor, 5-hydroxytryptophan with carbidopa [79].

HPRT is not directly included in the formation of dopamine, but its role in the formation of guanine-nucleotides, especially GTP, the substrate for the first and rate-limiting step in the synthesis of tetrahydrobiopterin (BH_4), could make for decrease in dopamine because BH_4 is the cofactor for tyrosine hydroxylase. Thus, limited local supply of GTP could lead to depletion of BH_4 and of dopamine. In fact, HPRT-deficient mice, which have low levels of striatal dopamine, have been found to have statistically significantly lowered striatal BH_4 [80]. Treatment of these mice with BH_4 did not correct the dopamine deficiency in mutant mice. Man is not a mouse and treatment was confined to one single day. In addition, Manzke and colleagues [81] treated a patient with a variant HPRT, in which the phenotype lacked abnormal behavior, with 17.5 mg/kg of BH_4 over 1 day. Levels of HVA and HIAA in the CSF, which were very low prior to treatment rose to normal with treatment; L-dopa plus carbidopa and 5-hydroxytrophan were given at the same time. The same treatment was given to a nine-year-old patient with classic Lesch–Nyhan disease and severe spasticity, who was reported to improve unequivocally, but the effect was lost after five weeks. It is unclear how much of the reported changes were related to BH_4.

In studies of adenosinergic systems in brain in HGPRT knockout mice, Bertelli *et al.* [82] found increased expression of the ADORA 1A adenosine receptor gene and a mild decrease in ADORA 2A expression.

TREATMENT

Allopurinol has been effective in reducing concentrations of uric acid and alleviating all of its direct clinical consequences. Doses of 200–400 mg/day lead promptly to normal plasma concentrations. Calculi and tophi are prevented or resorbed as concentrations of uric acid and in blood and urine fall. Nephropathy and arthritis are prevented. The total production of purine does not change; concentrations of xanthine and hypoxanthine increase. Some patients develop xanthine calculi. Determination of the levels of these oxypurines is very useful in providing the optimal dose of allopurinol. We aim to maximize the content of hypoxanthine without running the risk of oxypurinol lithiasis. It has become clear that doses required to accomplish this are highly individual and usually larger than employed in other diseases. An initial dose of 15–20 mg/kg is followed by assessment of concentrations of all of the oxypurines of the urine: uric acid; xanthine and hypoxanthine. Dosage is then modified, usually increased, to yield maximal concentrations of hypoxanthine which is soluble and minimal levels of xanthine, which causes the calculi in treated patients.

Dietary approaches to reduction of purine and uric acid output by reducing intake of purine-rich foods are ineffective. Similarly, feeding purines does not increase it. The production level appears set by the metabolism. On the other hand, these patients are virtually all thin and predominantly short. The enormous loss of nitrogen and preformed purine in the urine, along with dysphagia and vomiting, make keeping up nutrition difficult. A diet high in protein and calories appears prudent. The occurrence of megaloblastic anemia and the requirement of folate containing cofactors at two steps in purine synthesis would make the provision of folate prudent via folate rich foods or a supplement.

Pharmacologic approaches to therapy, based on the neurotransmitter imbalance, have not yet been successful, but this is a promising direction, possibly aided by PET and the demonstration of a reduction in dopamine transporters [77]. The availability of the cloned normal gene raises the possibility of gene therapy. Transfection of Lesch–Nyhan cells *in vitro* has been demonstrated, along with expression of normal enzyme [83]. Long-term expression *in vivo* remains an objective.

The only successful approaches to the self-injurious behavior have been the removal of teeth and physical restraint. Tooth removal can be selective. In addition to physical restraint, it is useful to be imaginative in finding ways to encourage purposeful activity to replace self-injurious behavior [84]. For instance, on entering an automobile, one adult patient in a community placement regularly managed to leave a hand in a place to be caught when the door was closed. His caretaker learned to avoid this problem by the simple expedient of asking the patient to close the door himself.

Treatment with S-adenosylmethionine has met with mixed results [85]. A majority of the patients we have seen experienced little benefit, and some patients clearly developed worsening of behavior. However, a few infants have experienced significant prolonged improvement in self injurious behavior.

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66

Adenine phosphoribosyltransferase (APRT) deficiency

MAJOR PHENOTYPIC EXPRESSION

Renal calculi and crystalluria, excretion of 2,8-dihydroxyadenine and deficiency of adenine phosphoribosyltransferase.

INTRODUCTION

Deficiency of APRT was first reported in 1976 by Simmonds and colleagues [1], and by Debray and colleagues [2], as a cause of urinary tract stones in children [1–3]. Initially, the calculi were mistaken for those of uric acid, and this is still a potential problem [4] when a routine chemical colorimetric reaction for uric acid is used to determine the compound being excreted in excessive quantity, or if lucency of the stone is considered diagnostic. An effective therapeutic response may compound under diagnosis [5]. The disease has most often been recognized in children, in whom urinary calculi are rare and thus more likely to trigger a search for an unusual cause, but the disease is now recognized in adults more commonly than in children [4]. This is particularly true in Japan, where the disease is more commonly encountered [6].

The enzyme APRT (EC 2.4.2.7.) (Figure 66.1) catalyzes the conversion of adenine to its mononucleotide (AMP). This enzyme and HPRT (Chapter 65) are purine salvage enzymes. The enzyme is the only route for the salvage of adenine. Deficiency is readily documented by assay of erythrocyte lysates. The gene has been localized to chromosome 16q24.3 [7–9] and has been cloned and sequenced. Mutations have been identified [10, 11], including three mutations that account for 96 percent of the mutant alleles found in Japan [12].

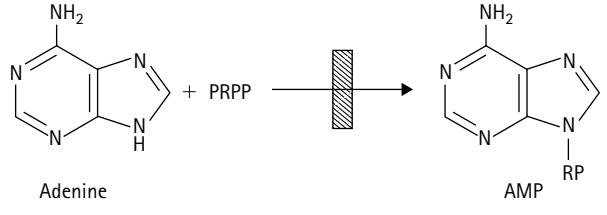

Figure 66.1 The reaction catalyzed by APRT. Magnesium is a cofactor. Abbreviations include PRPP: phosphoribosyl pyrophosphate and AMP, adenylic acid.

CLINICAL ABNORMALITIES

The clinical picture of APRT deficiency is entirely a function of the excretion of 2,8-dihydroxyadenine and its propensity to cause nephrolithiasis and nephropathy (Figure 66.2) [4, 13–16]. The severity of the disease is quite variable, ranging from no symptoms to life-threatening renal disease. A number of asymptomatic patients with APRT deficiency have been reported; they were found because they were screened family members of a known patient [4, 13, 14, 17]. Onset of symptoms has ranged from birth to 74 years [5, 13]. Rarely, the disease may be indicated by the presence of brown spots on the diaper.

Patients may have hematuria, dysuria, crystalluria or urinary tract calculi. The presenting complaint may be fever resulting from a complicating urinary tract infection. Calculi may also lead to renal colic or urinary retention. Obstructive crystals may lead to acute renal failure even in infancy. This may be reversible, but the long-term outcome in some patients is chronic renal failure leading to dialysis or renal transplantation [4, 15, 16, 18–22]. Deposition of crystals in the renal interstitium may lead to nephropathy and has been called DHA

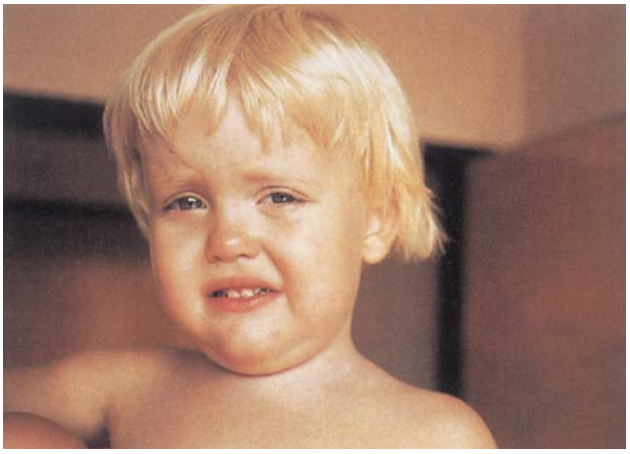

Figure 66.2 An 18-month-old with APRT deficiency who began passing stones at birth. At last report, he was a young, fit 24-year-old. (Illustration was kindly provided by Dr H. Anne Simmonds of the United Medical and Dental Schools, University of London.)

nephropathy [22]. On the other hand, some patients are asymptomatic.

Plain roentgenograms of the abdomen are usually negative in these patients since 2,8-dihydroxyadenine stones, like those of uric acid, are radiolucent; rarely, admixture of calcium will render these stones radio-opaque. Abdominal ultrasound or intravenous urography should be done if there is a suspicion of radiolucent stones. Calculi are usually thought first to be those of uric acid, and routine colorimetric analysis of the urine will not differentiate between uric acid and 2,8-dihydroxyadenine. A simple fluorescence method has been developed for the identification of 2,8-dihydroxyadenine stones. Calculi have been identified by a reddish-brown appearance when wet of a round friable stone, becoming grey when dry [23]. Mass spectrometry or scanning electron microscopy can be used for the correct identification of calculi [24].

It has become clear with increasing experience that there are no problems with other systems. Intelligence is normal. There is no abnormality of immune function.

GENETICS AND PATHOGENESIS

APRT deficiency is transmitted in an autosomal recessive fashion. Early reports of disease in heterozygotes have not held up. Consanguinity has been observed [3, 25, 26]. The disease has been divided into two subtypes: in type I, erythrocyte lysates have no activity, and in type II, there is residual activity. Now that it is possible to determine the mutation, these distinctions may become less useful, but homozygous type II patients have been found only in Japan; type I patients have been widely distributed, including in Japan [12, 27]. The frequency of a heterozygous allele for APRT deficiency has been given as 0.4 to 1.2 percent in various Caucasian populations [28–30], but the incidence of diagnosed homozygotes is much lower than would be expected from these figures. This would be consistent with the asymptomatic nature of some patients described.

The enzyme (Figure 66.1) is a dimer with a subunit molecular weight of approximately 19 kDa and 179 amino acids [31]. 2,6-Diaminopurine (DAP) and 8-azaadenine are substrates for the enzyme, as are many adenine analogs that are toxic to cells after conversion to their nucleotides. These compounds are used in selective media [32]. Cells from either type I or type II patients will not grow in azaserine-alanosine-adenine (AAA) medium, and they are resistant to the adenine analogs 6-methylpurine and 2,6-diaminopurine. APRT is widely distributed in tissues and, it is most commonly assayed in erythrocyte lysates, where normal activity is 16–32 nmol/h/mg hemoglobin.

In patients with the classic type I disease, there is less than 1 percent of control activity and immunoreactive protein [33–35]. Patients with residual enzyme activity of 10–25 percent of control in the type II group have displayed a variety of different properties, such as sigmoidal kinetics [27, 36], reduced affinity for phosphoribosylpyrophosphate (PRPP) and altered heat stability. Decreased amounts of immunoprotein have been found [37].

Heterozygotes for type I deficiency have activity in hemolysates approximating 25 percent of control. Levels of immunoreactive protein may be 22 percent to normal [33–35]. In contrast, in lymphoblasts or fibroblasts activity is 46 percent and cross-reacting material (CRM) 41 percent of control. This raises the issue of distinguishing type II homozygotes from type I heterozygotes. Testing for resistance of cultured cells to medium containing DAP or 6-methylpurine should resolve the question [28]. Documentation of the mutation is a better approach to these distinctions. In general, anyone with APRT activity of 25 percent or more will have no clinical symptoms.

The gene at 16q24 is the most telomeric gene on chromosome 16 [38]. It contains five exons and a 540 base-pair coding region [39, 40]. The promoter region, like that of other housekeeping genes contains no TATA or CCAAT boxes but contains 5GC boxes that are transcription factor binding sites [39]. A variety of mutations has been identified; many of them single-base substitutions. Many patients are compounds of two mutant alleles. A T insertion in intron 4 at the splice donor site has been found in 40 percent of the sizeable French series; it leads to abnormal splicing and loss of exon 4 in the mRNA [11, 14, 41–43]. The T insertion creates an MseI restriction site that is useful in diagnosis [43]. Another intron 4 splice donor site mutation, a G-to-T transversion, would also disrupt splicing. A relatively common mutation in Britain and Iceland was an A-to-T change in exon 3, which converts aspartate 65 to valine [11]. Five Icelandic patients were homozygous for this mutation. Some 55 families have been identified in Paris [41]. Only six pathologic alleles have been found in patients with inactivation of the APRT enzyme. A recent one of these is g.2098C > T in exon 5, which provides a stop codon at amino acid 147 (p.Gln 147X [44].

Among Japanese patients, the most common mutation is a T-to-C mutation in exon 5 which changes methionine 136 to threonine (M136T) [12, 45]. This has, to date, been found exclusively in type II patients. Actually, three mutations account for 95 percent of the mutant alleles in Japanese patients [12]. The M136T accounts for 67 percent. The other two are a G-to-A substitution which changed tryptophan 98 to a stop codon in patients with the type I phenotype [46, 47], and a four base, CCGA insertion in exon 3. Restriction fragment length polymorphism in this area has been useful in family and population studies, leading, for instance, to a prediction that the M136T mutation has been in existence since at least 300BC [48]. This mutation has also been referred to as APRT*J [48]. A partial deficiency of APRT has also been reported in a patient with the type II mutation on one allele and a null or type I mutation on the other allele [49]. Testing for the disease should be done by enzyme assay because 10 percent of mutations have remained unidentified [41].

The direct consequence of deficiency of APRT is the accumulation of adenine, which is oxidized in the presence of xanthine dehydrogenase to 2,8-dihydroxyadenine, which is very insoluble. The solubility of 2,8-dihydroxyadenine in water is less than 3 mg/L [50] but the compound may be supersaturated in urine. The excretion of adenine and 2,8-dihydroxyadenine occur in a ratio of 1:1.5. 8-Hydroxyadenine is excreted in a lesser amount. Concentrations of 2,8-dihydroxyadenine up to 80 mg/L (0.5 mmol/L) have been found in patients [50].

APRT deficiency has occurred in patients with Morquio syndrome [51]. The GALNS gene which is mutated in Morquio syndrome is located adjacent to APRT at 16q24.3, with APRT telomeric. A 100 Kb deletion was found. A Japanese patient was found [52] with a submicroscopic deletion at GALNS and APRT on one allele and a point mutation (R386C) in APRT on the other.

TREATMENT

Therapy is aimed at reducing the formation of 2,8-dihydroxyadenine by the use of a low purine diet and allopurinol [3, 15, 50, 53]. A dose of allopurinol of 5–10 mg/kg per day up to 200–300 mg per day in an adult has been reported to eliminate 2, 8-dihydroxyadenine from the urine [15]. Adenine still accumulates. Dosage of 300 mg twice daily has more recently been recommended [44]. In a patient allergic to allopurinol, Febuxostat in a dose of 80 mg daily led to significant reduction of crystalluria [54].

A high fluid intake is prudent. Alkali therapy is not beneficial; the solubility of 2,8-dihydroxyadenine is not altered by changes of urinary pH in the range obtainable physiologically. Stones already formed may be treated with lithotripsy [55–57]. Renal failure has been reported [58]. Patients have been treated with renal transplantation, but recurrence in the transplanted kidney has been reported, followed shortly by death [44].

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67

Phosphoribosylpyrophosphate synthetase and its abnormalities

MAJOR PHENOTYPIC EXPRESSION

Hyperuricemia, uricosuria, hematuria, crystalluria, urinary tract calculi, gouty arthritis, nephropathy, sensorineural deafness, and abnormal phosphoribosylpyrophosphate synthetase, in which activity is greater than normal.

INTRODUCTION

Accelerated activity of phosphoribosylpyrophosphate (PRPP) synthetase associated with overproduction of uric acid and gout was first reported in 1972 by Sperling and colleagues [1, 2]. A small number of patients have now been identified, establishing the relationship with hyperuricemia [1–10]. In some kindreds, there has been sensorineural deafness [11–13].

5-Phosphoribosylpyrophosphate synthetase (EC 2.7. 6.2) (Figure 67.1) catalyzes the initial step in the *de novo* synthesis of purines in which ribose-5-P reacts with adenosine triphosphate (ATP) to form PRPP. The PRPP formed, provides the substrate for the first rate-limiting step in the ten-step purine *de novo* pathway. Increased quantities of intracellular PRPP lead to overproduction of purine *de novo* and of uric acid. PRPP synthetase is coded for by two genes on opposite arms the X chromosome at Xq22-24 and Xp22.2-22.3 [14, 15]. The genes have been cloned and sequenced [16] and referred to as *PRPS1* and *S2* [17]. A small number of point mutations have been defined in *PRPS1* in patients with overactivity and altered allosteric properties of the enzyme.

In six patients with overactivity of PRPP synthetase gout and/or uric acid calculi but without neurologic abnormality,

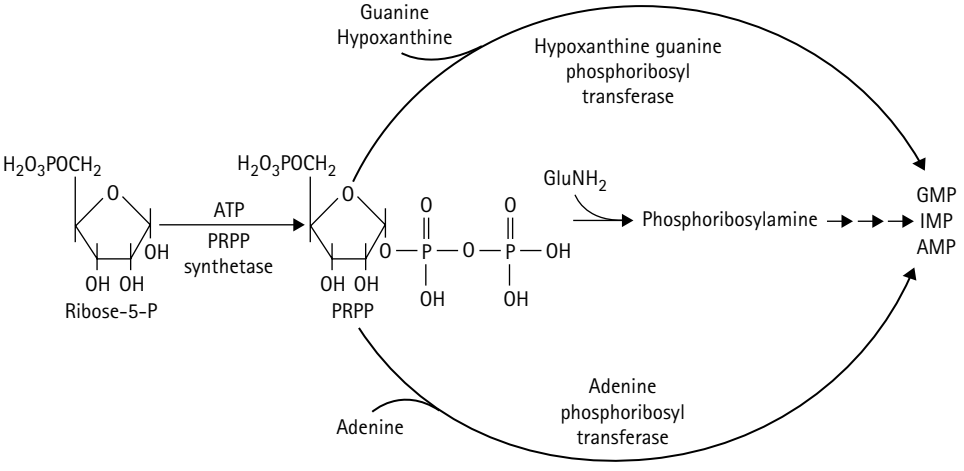

Figure 67.1 PRPP synthetase. The role of the product PRPP is central in the interrelation of purines and their nucleotides.

no mutation in the cDNA of *PRPS1* or *S2* were found; instead, there were increased quantities of the S1 isoform whose physical and catalytic properties were normal [18]. A genetic etiology for this overexpression has not been discovered.

CLINICAL ABNORMALITIES

The invariant clinical features of this disease are hyperuricemia and uricosuria. Therefore, a patient is subject to any of the clinical consequences of the accumulation of uric acid in body fluids. Gouty arthritis has been reported with onset as early as 21 years of age [1]. Renal colic has been observed, as well as the passage of calculi [3]. One boy developed hematuria at the age of two months and was found to have crystalluria, hyperuricemia, and uricosuria [4]. In families with this early onset phenotype, females have manifestations of hyperuricemia prior to menopause [13].

A small number of families have been reported [5, 6, 11, 12] in which deafness has been associated with an abnormally active PRPP synthetase. In one family, there were three involved males, each of whom also had severe neurodevelopmental impairment. The mother had high-tone deafness. A large kindred had previously been reported in which there were X-linked deafness and hyperuricemia; an enzyme defect was not demonstrated at the time of report [19].

Another patient [5] initially appeared to have impaired mental development and his behavior was thought to be autistic (Figure 67.2). With time, it became apparent

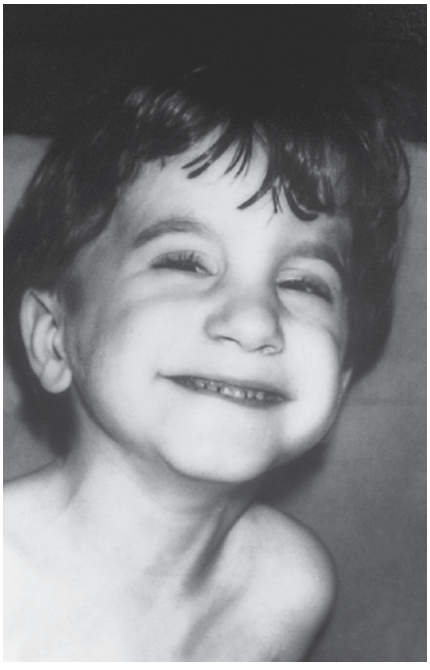

Figure 67.2 SM: A three-year-old with an abnormal PRPP synthetase. The odd grimace was characteristic. (Reprinted from the *Journal of Pediatrics* [5] with permission from Elsevier.)

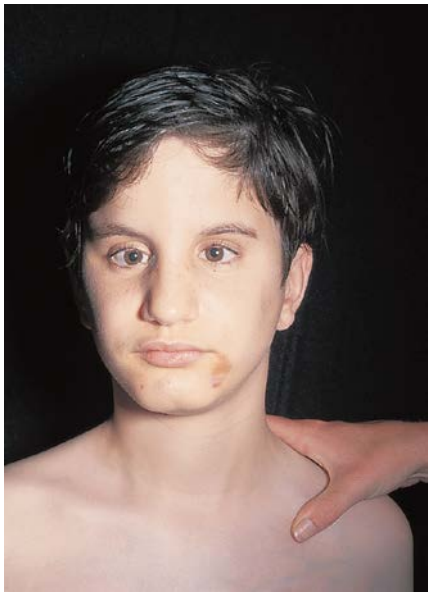

Figure 67.3 SM: At 14 years of age.

that he was deaf and his behavior was quite appropriate (Figure 67.3). As an infant, he failed to cry with tears and was found to have absent lachrymal glands. Other structural anomalies were a glandular hypospadias and hypoplastic teeth. The relationship of all of these problems to the metabolic abnormality is not clear, but the mother also had the abnormal PRPP synthetase and hearing loss, and she and her father had problems with behavior.

Patients with late teenage or young adult gout or urolithiasis have been exclusively male and have had no neurologic abnormalities [10, 13]. These patients have had overabundance of the normal S1 isoform.

All these patients have had increased amounts of uric acid in the blood and urine. In our patient, prior to treatment, the concentration in serum ranged from 8.5 to 11.6 mg/dL [5]. Urinary excretion ranged from 1.84 to 3.26 mg/mg creatinine. In the initial proband [1], uric acid excretion was 2400 mg per 24 hours. Overproduction of purine *de novo* was documented [5] by measuring the *in vivo* in conversion of ^{14}C-glycine to urinary uric acid. In seven days, 0.7 percent of the isotope of glycine administered was converted to uric acid – seven times the control level of 0.1 percent.

GENETICS AND PATHOGENESIS

The S1 isoform of PRPP synthetase, while coded for by a gene on the long arm of the X chromosome [14] may be fully recessive [1] or may be expressed in the heterozygous female [3]. This could reflect different degrees of Lyonization. On the other hand, it is easier for an overactive enzyme, rather than the more common deficient one encountered in inborn errors, to function as an X-linked dominant.

The *PRPS1* gene has been localized to Xq22-24 [15, 20]. The human PRPP *S1* gene spans 30 kb and has 17 exons;

the cDNA encodes a transcript of 2.3 kb and a protein of 317 amino acids. The *PRPS2* gene is located at Xp22.3-22.2 [15, 21] on the other end of the chromosome. Regardless of the location, the two genes have over 90 percent homology in the cDNA and amino acid sequence.

The *PRPS2* gene codes for a transcript in testis, a 318 amino acid sequence [22–24]. The *PRPS1* gene is located between the α-galactosidase (*GLA*) and *HPRT1* genes [14]. The *PRPS1* and these two genes both undergo inactivation with Lyonization [25]. The *PRPS2* gene is situated between two genes on the short arm that escape inactivation distally and ZFX proximally. The *XIST* gene is transcribed only from the inactive X chromosome. A third gene (*PRPS3*) maps to chromosome 7 and is expressed only in testis [22].

In six male patients with overactivity of PRPP synthetase and resistance to purine nucleotide feedback, there was a single-base transition, which led to a single amino acid change at positions 51 (D51H), 89, 113 (N113S), 128 (L128I), 182 (D182H), and 192 (H192Q) [16, 17].

The molecular basis of the disease in the patients with superactive enzyme activity is an altered PRPP synthetase structure. Activity may be three times that of the normal enzyme [3]. In one of the families studied, increased enzyme activity was demonstrable only at low concentrations of phosphate, and there was diminished responsiveness to feedback inhibition by purine nucleotides [2]. In another family, an elevated level of enzyme-specific activity was demonstrable over a wide range of phosphate concentrations, and feedback inhibition was normal [3]. The amounts of immunoreactive enzyme protein were normal [26]. These observations indicate the presence in normal amounts of a protein in which structural alteration leads to increased specific activity. The data are consistent with the presence of two important sites on the enzyme: a catalytic site altered by one mutation and a regulatory site altered by the other. In one patient, the altered structure affected both catalytic and regulatory sites [6]. The enzyme may have increased affinity for the substrate ribose-5-phosphate [27].

In addition to its other properties, the PRPP synthetase in one patient had diminished stability to heat [6]. Distinctly diminished levels were found in old as opposed to young erythrocytes obtained by density separation. For these reasons, the activity of PRPP as determined in erythrocyte lysates was not elevated, but in fact was lower than normal. This same observation was made in another family in which erythrocyte levels of PRPP were not elevated [11]. In the first patient, the activity of PRPP synthetase in hemolysates was less than 10 percent of normal at concentrations of inorganic phosphate over 1 mM. In this sense, a direct assay that indicates a deficiency of the erythrocyte's enzyme could be the clue to the presence of an abnormal enzyme that is superactive *in vivo*. Nevertheless, in a patient who clinically appears to be a candidate for a diagnosis of PRPP synthetase overactivity, a normal result of an erythrocyte assay should be followed up by an intact cell method.

Intact cultured fibroblasts can be shown to incorporate each of the purine bases, adenine, guanine, and hypoxanthine, more rapidly into nucleotides than do controls [6]. Adenosine conversion to nucleotide is normal. Incorporation of ^{14}C-formate into formylglycinamide ribotide (FGAR) in the presence of azaserine is also accelerated. These findings indicate the presence of increased intracellular concentrations of PRPP. Concentrations can be measured in fibroblasts or lymphoblasts and found to be elevated [6].

The single amino acid substitutions in the S1 enzyme are clearly scattered over much of the protein; yet they all lead to decreased responsiveness to feedback inhibition by adenosine diphosphate (ADP) and guanosine diphosphate (GDP) [16]. Also, the binding of MgATP to the active site is normal. The mechanism for the allosteric changes resulting in superactivity is unknown, but it must be structurally diffuse. Mechanisms for the increased transcription in the patients in whom no structural changes were found are even less clear, although a number of possibilities have been excluded [28].

Moderate deficiency of activity of PRPP synthetase and mutations in PRPS1 were found in patients with a triad of peripheral neuropathy, hypotonia, and deafness [29]. These patients had previously been referred to as X-linked recessive Charcot-Marie-Tooth disease or Rosenberg-Chutorian syndrome. Severe deficiency of activity of the enzyme and hypotonia are found in Arts syndrome [30] an X-linked syndrome of impaired mental development hypotonia, ataxia, optic atrophy, and peripheral neuropathy. Missense mutations in PRPPS and mild deficiency of activity of the enzyme are found in patients with X-linked nonsyndromes features deafness [31].

Pathogenesis has been related to low levels of the important nucleotide GTP which is essential for G-protein function and synthesis of dopamine and pterins in brain [32, 33]. Erythrocyte levels of the pyridine nucleotide NAD are also low [34]. Purine nucleotide depletion appears also to lead to starvation of neurons for ATP.

TREATMENT

Allopurinol is the treatment of choice in overproduction of hyperuricemia. Treatment of abnormalities in PRPP synthetase is simpler than that of hypoxanthine guanine phosphoribosyl transferase (HPRT) deficiency, because in the presence of normal HPRT activity, there is extensive reutilization of hypoxanthine accumulating behind the block in xanthine oxidase, leading to a substantial decrease in the overall excretion of oxypurines in the urine. In contrast, in HPRT deficiency, there is simple substitution of hypoxanthine or xanthine for uric acid, and the total oxypurine excretion does not change. Hearing should be tested promptly and appropriate intervention provided. Treatment of ATP starvation with adenosine is not an option, because adenosine is not absorbed by the intestine. S-adenosylmethionine, which crosses the intestine and the blood-brain barrier, is a potential therapeutic solution. It

has been reported to be somewhat effective in two patients with Arts syndrome [35].

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68

Adenosine deaminase deficiency

MAJOR PHENOTYPIC EXPRESSION

Severe combined immunodeficiency disease, involving immunoglobulins and cell-mediated immunity; clinical immunodeficiency triad of persistent diarrhea, progressive pulmonary disease, and extensive moniliasis; skeletal abnormalities; and deficiency of adenosine deaminase.

INTRODUCTION

The discovery of the association between adenosine deaminase (ADA) (EC 3.5.4.4) deficiency and severe combined immunodeficiency disease in the early 1970s [1] provided exciting evidence of metabolic causation of immunodeficiency. This established a relationship between the metabolism of purines and developmental immunobiology, and this was reinforced by the discovery of purine nucleoside phosphorylase deficiency.

The discovery of this disorder was an interesting example of important observations made by a prepared mind. Giblett, a pediatric pathologist [1, 2], was reviewing polymorphic markers in candidates for bone marrow transplantation and found one deficient in ADA; a sample from a second unrelated patient studied one week later, was also ADA-deficient. Within one year, a number of immunodeficient patients with ADA deficiency were reported [3]. Giblett and colleagues [4] used the same approach to discover purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) deficiency.

ADA and PNP deficiencies represent enzymatic defects in the metabolism of purines that primarily affect cells of the immune system. The mechanism appears to be the accumulation of purine nucleotides which are toxic to T and B cells [5]. ADA is an enzyme of purine interrelations, which converts adenosine to inosine (Figure 68.1). This is an important reaction because adenosine is not a substrate for nucleoside phosphorylase, which converts inosine and guanosine to hypoxanthine and guanine. ADA is widely distributed in animal tissues. This enzyme is determined by a gene on chromosome 20q13.11 [6]. The gene for ADA has been cloned and sequenced [7]. A considerable number of mutations have been identified [8–10], most of them single amino acid changes.

Mutated alleles have been classified into five groups [9]. Those with deletions and nonsense mutations are in group I with mutations that conferred less than 0.05 percent of ADA activity and led to the SCID phenotype. In contrast, alleles in group II had delayed onset immunodeficiencies. Group III expressed delayed late, or partial phenotypes. Those in group IV had partial deficiency and were identified by newborn screening or having healthy relatives of patients.

Patients with deficiency of adenosine deaminase 2 (ADA2) have had a complex immunogic and vascular phenotype.

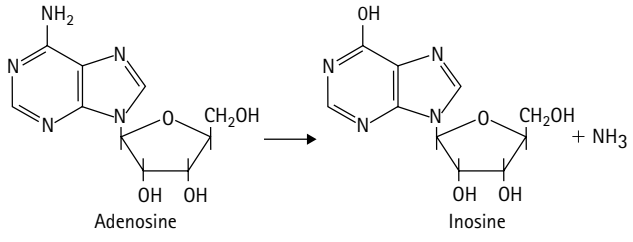

Figure 68.1 The ADA reaction.

CLINICAL ABNORMALITIES

Classic patients with ADA deficiency have a distinct syndrome of severe combined immunodeficiency disease (SCID). In common with other patients with SCID, they have both defective immunoglobulins or bone marrow-derived B-cell function and defective cell-mediated immunity or thymus-derived T-cell function. Patients with B-cell or humoral immunodeficiency have infections caused by organisms such as the pneumococcus with capsules, as well as some viruses. Patients with T-cell or cell-mediated immunodeficiency have infections caused by opportunistic organisms, such as monilia, or by viruses. Patients with combined immunodeficiency have both types of infection.

Most patients with SCID present in the first months of life with failure to thrive and recurrent or persistent diarrhea [1, 3, 11–14]. The diagnosis is not usually made until the onset of infection that follows the disappearance of maternal antibody. Severe bacterial and viral infections occur very early in life. A majority of patients have had extensive candidiasis. Many have presented in the first weeks or months of life with thrush, diarrhea, and pneumonia. Many have had bacterial infections of the skin. Recurrent otitis media is common, and pneumonia is a frequent complication. Many patients have died, often of infections with opportunistic organisms, some viral and some bacterial, that are not usually productive of severe infections in ordinary individuals.

Isolated defects of the cellular immune system, as well as severe forms of combined immunodeficiency, have been described in 10–15 percent of individuals with ADA deficiency [6, 15]. These patients have had a milder course of disease, often with a later onset [9]. A number of patients have been observed with milder forms of the disease reflecting higher levels of residual ADA activity. Adult-onset immunodeficiency was reported in two sisters with a residual ADA activity of 5–13 percent [16]. Furthermore, ADA deficiency has been described without abnormality in immune function [9, 17]. A tandem mass spectrometry (MS/MS) method for the detection of ADA deficiency has been developed [18]; so neonatal screening is now possible, and this should lead to complete ascertainment and better definition of the clinical spectrum. In general, it appears that immune function may be normal if levels of ADA are over 5 percent of control.

Patients with ADA deficiency, like those with other types of SCID, are at risk for the development of graft-versus-host disease if they receive blood transfusion from a donor with immunocompetent T cells. They are also at risk for disseminated diseases following immunization with live attenuated vaccines, such as poliomyelitis. Active infection has occurred following administration of oral polio vaccine. Known patients are better immunized for this disease with Salk inactivated vaccine.

The majority of patients reported with ADA deficiency have been infants with SCID and recurrent infections [19, 20]. Pneumonia may be caused by *Pneumocystis carinii* or by viruses. Candidiasis may involve the skin, the mucosa of the mouth, esophagus, or vagina, and stool cultures may reveal this organism. Patients surviving infancy may have pulmonary insufficiency, a consequence of repeated infection. Among late-onset presentations were pulmonary insufficiency and recurrent warts in adult sisters [21].

Autoimmune disease is also a feature of ADA deficiency. Presentations have included autoimmune thyroid insufficiency [22, 23], autoimmune thrombocytopenia [24], and fatal autoimmune hemolytic anemia [25].

Neurologic abnormalities may occur in ADA deficiency; they are more common in PNP deficiency. They may be a consequence of infection or possibly of the severe failure to thrive. However, in some patients, neurologic abnormality has appeared to be a metabolic consequence of the disease. Patients have had spasticity, nystagmus, tremors, dystonic posturing, athetosis, hypotonia, and head lag [20, 26–28]. In at least one patient, improvement was documented after successful treatment with enzyme replacement [26]. Neurologic problems are not present in early infancy; their severity appears to increase with age. Developmental delay has been reported to be more prevalent in ADA-deficient patients with SCID than in ADA-normal patients with SCID [20]. In a similar study of patients with SCID treated with bone marrow transplantation [29], tests of cognitive function revealed no differences between the two groups, ADA-deficient and non-ADA-deficient. However, among the ADA-deficient patients, there was significant inverse correlation between the levels of deoxyadenosinetriphosphate (dATP) at diagnosis and IQ. In behavioral assessment, the ADA-SCID patients functioned in the pathologic range in all domains, while the non-ADA patients scored in the normal range. Neurologic examinations were unremarkable in these patients, and none had seizures.

Among neurologic complications, a high incidence of bilateral sensorineural deafness has been observed [28, 29]. In a report of 12 patients with ADA deficiency successfully treated with bone marrow transplantation, 58 percent had bilateral deafness [30].

Hepatic dysfunction has been observed in this disease [31, 32] and many patients have elevated serum transaminase levels; levels have improved with replacement therapy. Recurrent hepatitis has been followed by chronic hepatobiliary disease. B-cell lymphomas have been related to infection with Epstein–Barr virus.

Physical examination may be remarkable only for evidence of infection, poor growth, and failure to thrive. Absence of lymphatic tissue may be striking, and this may be evident on palpation or examination of the pharynx. It is often first recognized roentgenographically. In roentgenograms, the upper mediastinum is narrow. In lateral views, there may be retrosternal radiolucency and no thymus shadow can be seen. Examination of the blood reveals profound lymphopenia [32]. Total lymphocyte count may be less than 500 per mL. Most of these patients have

chronic pulmonary changes such as those seen in infections with *Pneumocystis carinii.*

A bony dysplasia is an impressive feature of the syndrome [33]. The sacroiliac notch may be large, as in achondroplasia and the ilium flares outward, resembling Mickey Mouse ears. The acetabular angle is reduced, also like that of achondroplasia. The pubis is short and the ischium squared off. The ribs are flared, enlarged anteriorly, and cupped at the costochondral ends, resembling changes seen in rickets. In the spine, there is platyspondyly and an appearance reminiscent of mucopolysaccharidosis. In contrast to the spine in achondroplasia, the interpedicular distance in these patients does not decrease from L1 to L5. Growth arrest lines may be unusually thick. Roentgenograms of the bones may reveal profound osteoporosis. One patient had compression fractures of two vertebral bodies.

The ADA2 phenotype displays autoimmune disease, lymphoproliferation and combined immunodeficiency. Patients often have lymphadenopathy, hepatosplenomegaly, and anemia. Ulcerative bowel disease has been observed leading to enterectomy. Defective T-cell function, elevated levels of IL-6 and very low TNα are present. Vasculopathy and inflammation have been observed without clinical or routine biochemical signs of inflammation [34].

Adult-onset disease is increasingly being recognized [35] with recurrent infections with opportunistic organisms, such as oral and vaginal candidiasis or viral warts, as well as commonly pathogenic organisms causing tuberculosis for example. A patient was reported with Burkitt lymphoma following successful treatment with polyethylene glycol-linked ADA (PEG-ADA) [36].

Skin tests for delayed hypersensitivity are deficient and skin tests for candida are negative in patients known to have had candidal infection. Skin tests for streptokinase and other antigens are negative. The response of lymphocytes *in vitro* to phytohemagglutinin and other lectins is reduced or absent, and the formation of T-cell rosettes is poor. All immunoglobulins in most of these patients are decreased in concentration, once the infant is old enough to have lost immunoglobulin transferred from the mother. These include IgG, IgM, IgA, and others, but often it is to a variable degree of each. The antibody response to the injection of an immunizing antigen is faulty.

Pathologic examination of the thymus at autopsy has revealed a very small organ with little differentiation into lobules. No Hassall's corpuscles were seen. There was no central medullary area and no differentiation into cortex and medulla. Huber and Kersey [37], who analyzed tissues from four patients with ADA deficiency and five without, all of whom had died of combined immunodeficiency disease, believed that they could distinguish between the two groups. The patients without ADA deficiency appeared to have failed to develop thymic tissue in early embryonic life, whereas the ADA-deficient patients had what they called "extreme involution". The thymus in these patients appeared to have known better days.

GENETICS AND PATHOGENESIS

ADA deficiency is transmitted as an autosomal recessive disease. In heterozygous carriers for ADA deficiency, levels of enzyme activity are about half the normal level [5, 38–40], but detection of carriers by enzyme assay in erythrocytes or fibroblasts is not reliable. Polymorphism in the ADA cDNA has been successfully used for this purpose and, of course, in a family in which the mutation is known, this is a reliable approach to carrier detection. The incidence of the disease from neonatal screening in New York approximated one in 400,000–500,000 births [19, 41]. ADA deficiency accounts for 15 percent of the patients with SCID and one-third of patients with autosomal recessive SCID [42]. Prenatal diagnosis has been accomplished by assay of the enzyme in cultured amniocytes and chorionic villus samples [38, 43, 44].

The molecular basis of the disease is the deficiency of the activity of ADA, a 41-kDa single polypeptide chain enzyme, the N terminal of which is post-translationally removed to yield 332 amino acids [45]. The *ADA* gene [7] consists of 12 exons spanning about 32,000 bases of DNA. Analyses of the *ADA* genes isolated from patients with ADA deficiency have revealed a heterogeneous pattern of mutations as causes of deficient enzyme activity [8, 9, 46–49]. Most patients are compounds of two mutant genes. Severe combined immunodeficiency and low levels of mRNA and protein have been seen with a 5-bp deletion in exon 10 [46] and a glycine 20 to arginine point mutation, while later-onset, milder disease was found with a point mutation changing arginine 253 to proline [49] and substitution at 156 of histidine for arginine. Splicing and other missense mutations have been identified [47]. The same mutation found in more than one family has often arisen at CpG hot spots [50, 51]. A329V, a relatively common mutation has been found in a number of unrelated patients [8]; as has R211H. Most deletions are small, but may introduce stop signals. Splicing site mutations, such as a G-to-A change in IVS10, which inserts 100 amino acids, have been observed in patients with more indolent disease, suggesting that alternate splicing may provide useful amounts of the wild-type enzyme [52, 53]. In a patient with late-onset SCID diagnosed at the age of 16 years, a homozygous mutation in intron 11 and an 11p deletion of adjacent base pairs suppressed aberrant splicing, and T and B cells had 75 percent of normal ADA activity, and ADA protein of normal size indicating somatic reversion [10]. Prenatal diagnosis has been accomplished by the assessment of mutation in the ADA gene [54].

Mutations that have led to severe SCID included 20 mutations clustering around the active site [9]; mutations consistent with normal immune function included R142Q, R149Q, A215T, G239S, and M310T [9].

ADA catalyzes the irreversible deamination of adenosine (see Figure 68.1) to form inosine. Deoxyadenosine also serves as a substrate for the enzyme. Intracellular adenosine is produced in the catabolism of RNA and also by the

hydrolysis of S-adenosylhomocysteine, an intermediary in transmethylation reactions [55].

ADA may be assayed in the erythrocyte by means of a technique that measures ammonia liberated from adenosine. The enzyme in intact erythrocytes of normal individuals had a mean activity of 0.29 nmol/min per mL packed cells. There has been no detectable ADA activity in most patients studied [5, 39, 40]. A screening test has been developed [56] which permits the diagnosis on spots of dried blood on filter paper and is employed in neonatal screening.

In ADA deficiency, there is accumulation of adenosine and deoxyadenosine. Normal plasma concentrations of adenosine are 0.05–0.4 mM/L; levels of deoxyadenosine are below the level of detection. In ADA-deficient patients, plasma concentrations of adenosine and deoxyadenosine range from 0.5 to 10 mM/L. Large amounts of deoxyadenosine are excreted in the urine.

Inhibitors of ADA are toxic to cells. The pathophysiologic mechanism by which ADA deficiency produces immunodeficiency appears to be the consequence of the accumulation of adenosine and deoxyadenosine, which are converted to ATP and dATP. ATP and, especially, dATP have been shown to be toxic to lymphoid cells of the immune system [6, 57–60]. It is thought that accumulated dATP inhibits ribonucleotide reductase, which catalyzes the conversion of ribonucleotide diphosphates to deoxyribonucleotide diphosphates and in this way inhibits the synthesis of DNA [59]. Consistent with this hypothesis, deoxynucleosides of cytosine, thymine, and guanine are capable of reversing the toxic effects of the ADA inhibitor, deoxycoformycin [60], but there appear to be many possible mechanisms of the deficiency of immune function. Deoxyadenosine itself also leads to chromosomal breakage through inhibition of DNA repair. ADA activity is highest in lymphoid tissues which have high rates of turnover, consistent with lymphotoxic effects of ADA deficiency [61].

ADA inhibitors, coformycin and deoxycoformycin, produce a metabolic pattern in normal cells similar to that of ADA-deficient cells, and so does an inhibitor of nucleoside transport; neither compound had any effect on ADA-deficient cells [60]. Lymphocytes and lymphoblasts undergo apoptosis when treated with deoxyadenosine [62]. The activity of S-adenyosylhomocysteine (SAH) hydrolase is reduced in ADA deficiency, a consequence of suicide-like inactivation by deoxyadenosine [64]. Accumulation of adenosylhomocysteine could inhibit transmethylation.

Adenosine also serves as a regulator of blood flow and an inhibitor of platelet aggregation, lipolysis, and neurotransmitter release. It modulates beta-adrenergic receptor and insulin-mediated responses; it stimulates steroidogenesis and histamine release; and it inhibits superoxide and hydrogen peroxide release from neutrophils [65]. It is not clear that any of these functions is altered in ADA deficiency.

Newborn screening for this disease, and other causes of SCID, has been developed in dried blood spots [66] and incorporated into state programs for newborns. The method employs polymerase chain reaction to detect T-cell receptor excision circles (TRECs) that are pieces of DNA that occur during T-cell development.

TREATMENT

The current definitive treatment of ADA deficiency is bone marrow transplantation. The first survivors of SCID due to ADA deficiency were those that had been successfully treated with bone marrow transplantation [67] or with transplantation from fetal liver (Figures 68.2 and 68.3). Successful treatment by bone marrow transplantation has readily been accomplished when a histocompatible donor has been available; or, in the absence of such a

Figure 68.2 MR: A three-month-old boy with combined immunodeficiency. He had failed to thrive, had sparse facial and scalp hair, and had begun to have repeated infections. (Courtesy of Dr R Keightly of the University of Alabama.)

Figure 68.3 MR: At ten months of age, nine months following transplantation with fetal liver. He was thriving and looked well. (Courtesy of Dr R Keightly of the University of Alabama.)

donor, with half-matched parental marrow after removal of most of the post-thymic T cells [29]. In the presence of histocompatibility antigens (HLA)-identical bone marrow, the engraftment rate is around 80 percent, and full immune repopulation occurs in approximately six months [66]. In experience with patients with SCID, four of whom were ADA-deficient, all received HLA-identical bone marrow grafts and survived [68].

It has been found that transfusion of frozen irradiated red blood cells from normal individuals provided circulating levels of ADA and restored normal and cell-mediated immunity [27, 69, 70]. Levels of dATP were reduced [70]. The half-life of transfused ADA activity is 30 days and so treatment must be repeated every four weeks.

These observations led to the development of enzyme replacement therapy using bovine ADA conjugated to polyethyleneglycol (PEG-ADA), which has proved to be useful for many patients [24, 69–72]. It is initially given twice a week and later weekly as an intramuscular injection. PEG-ADA treatment restores immune competence. PEG-ADA has been employed to prepare very ill patients for transplantation.

Following successful transplantation survivors have been noted [73] to develop neurologic, cognitive and behavioral abnormalities. This has not been observed in patients without ADA deficiency treated with bone marrow transplantation.

ADA deficiency has also been treated by gene therapy [74, 75]. Cells isolated from autologous peripheral blood of the patient's cord blood have been used as recipients for transfer of a viral vector containing the human ADA gene and then infused into the patient. In others, treatment was with repeated infusions of transduced peripheral mononuclear cells or stem cells from the marrow. Patients have now been reported [76, 77] with successful reconstitution of immunity in ADA deficiency in the absence of PEG-ADA with follow up of two and four years. Two patients were successfully treated with stem cell gene therapy without cytoreductive conditioning [78]. Recovery of immunity was partial; it was also temporary, but lasted six and ten years. In two other patients in whom enzyme therapy was employed, treatment with stem cell gene therapy without cytoreductive conditioning led to sustained engraftment and improved in immune function [79].

In a patient with ADA2 deficiency homozygous for p.Arg169Gen stem cell transplantation rescued the immunodeficiency phenotype and may have prevented the development of vasculopathy [34]. He was well at follow up five years later. Some patients had a significant response to treatment with etanercept.

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69

Adenosine kinase deficiency

MAJOR PHENOTYPIC EXPRESSION

Developmental delay, seizures, frontal bossing and dysmorphic features, macrocephaly, muscular hypotonia, hepatic steatosis and fibrosis, recurrent hypoglycemia, hyperinsulinism, failure to thrive and short stature, congenital cardiac anomalies, megaloblastic anemia, intermittent hypermethioninemia, increased S-adenosylmethionine and S-adenosylhomocysteine, and mutations in the *ADK* gene.

INTRODUCTION

Adenosine kinase (ADK) deficiency was first described in 2011, the result of an exome sequencing study in six patients from three families with global retardation, epilepsy, hepatic dysfunction, dysmorphic features and hypermethioninemia [1]. Increased urinary concentration of adenosine was considered to confirm the diagnosis. Since then, eleven additional patients from eight families were published [2], and one report of a family (three girls) with previously undetermined hypermethioninemia [3] could be assigned as ADK deficiency [2], so in total 20 patients have been published. All patients share the clinical features of psychomotor retardation, muscular hypotonia, and frontal bossing. All but one family presented with hepatic disease and most patients developed severe epilepsy in infancy.

ADK converts adenosine to adenosine monophosphate (AMP) (Figure 69.1). Direct phosphorylation of adenosine by ADK is a major route for the recycling of adenosine. Defective activity of the enzyme leads to accumulation of adenosine. It disrupts the methionine cycle by reversing the balance of S-adenosylhomocysteine hydrolase (SAHH) leading to elevated levels of S-adenosylhomocysteine (AdoHcy), S-adenosylmethionine (AdoMet), and methionine.

CLINICAL ABNORMALITIES

The first patients with *ADK* mutations identified were two Swedish siblings with global developmental delay and mild hepatic dysfunction [1]. Both had failure to thrive from infancy and dysmorphic features including macrocephaly, frontal bossing, hypertelorism, and slender hands and feet. They developed "sparse or absent" language and had progressive weakness and wasting of muscle. Seizures began before three years of age and were poorly controlled with anticonvulsant medications. EEG revealed slow background activity with diffuse multifocal discharges and spike slow–wave complexes resembling Lennox-Gastaut. The girl died during sleep at ten years, her brother at the age of 25 years (personal communication A. Wedell).

Four Malaysian patients from two unrelated families with similar biochemical and clinical phenotypes were identified thereafter [1]. Their parents were first cousins. The patients had severe developmental delay and seizures. All but one had macrocephaly and frontal bossing. Three had cardiac anomalies: one, pulmonary stenosis and a secundum septal defect; one had a septal defect, and one mild coarctation of the aorta. Two had mild sensorineural hearing loss. ADK deficiency has now been delineated as a complex disease of mainly encephalopathy, dysmorphic features, and abnormal hepatic function, but also recurrent hypoglycemia, congenital heart defects, failure to thrive and short stature, megaloblastic anemia, and cholelithiasis [1, 2]. The spectrum of the disease is illustrated by three patients from two related consanguineous Turkish families. The first girl was symptomatic from birth with hypoglycemia (blood glucose not detectable) along with hyperinsulinism, hyperbilirubinemia, and hypothermia (35.1°C). Hyperinsulinism could be controlled with

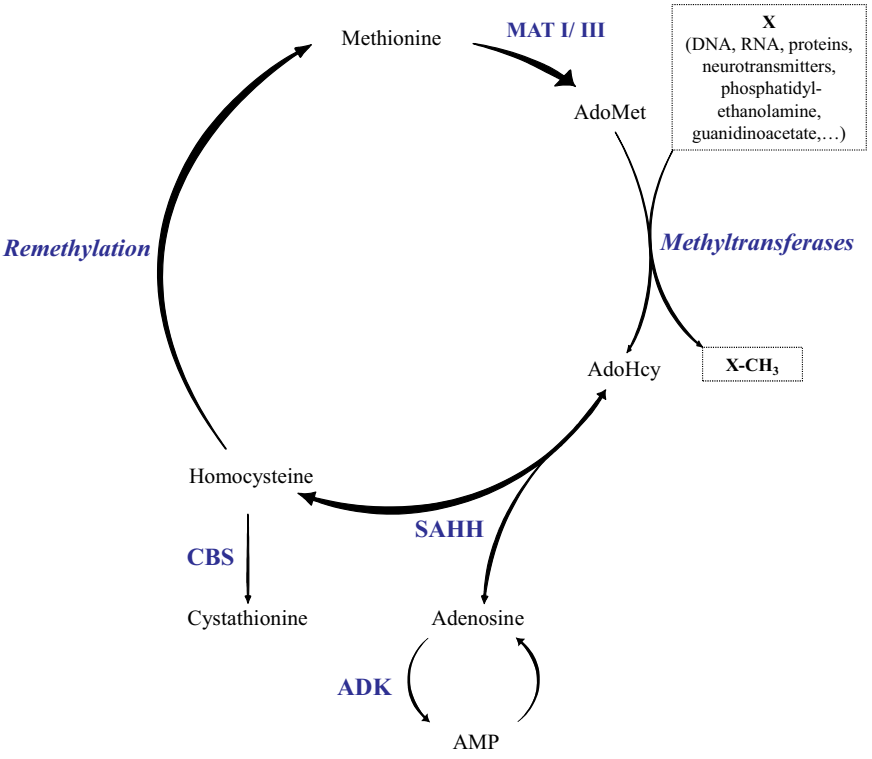

Figure 69.1 The adenosine/AMP futile cycle and its relationship to the methionine cycle. Abbreviations include: AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; AMP, adenosine monophosphate; MAT I/III, methionine adenosyltransferase; SAHH, S-adenosylhomocysteine hydrolase; ADK, adenosine kinase; CBS, cystathionine β-synthase.

diazoxide. Methionine concentrations were only mildly and intermittently elevated (max. 107 μmol/L, N < 45), whereas in plasma and cerebrospinal fluid (CSF), concentrations of AdoMet and AdoHcy were markedly and consistently high (plasma: AdoMet 355 nmol/L, n 71-118; AdoHcy 224 nmol/L, n 9.3-14; CSF: AdoMet 384 nmol/L, n 104-337; AdoHcy 218 nmol/L, n 5-31). Echocardiography had revealed a persistent ductus arteriosus and persistent foramen ovale, and a perimembranous ventricular septum defect that needed cardiosurgical correction because of hemodynamic decompensation at the age of four months. In addition to hepatomegaly, epilepsy developed at five months of age, and severe muscular hypotonia without head control, severe global developmental delay and frontal bossing were obvious. At the age of 15 months, she died as a result of a pulmonary infection. Her younger brother (Figure 69.2) also presented from birth with hypoglycemia and hyperinsulinism, muscular hypotonia, cholestatic hyperbilirubinemia, increased transaminases, severe liver dysfunction, and hepatomegaly. Hypermethioninemia was detected (methionine 176 μmol/L at day 6; 400 μmol/L at day 34) but spontaneously became normal thereafter as did liver function. The report for the nine-year-old boy was a happy and cooperative boy. He was severely retarded, had a hypotonic-dystonic movement disorder with changing muscle tone and no speech, but he communicated with sounds and gesticulation. He was nearly blind as a result of retinal dystrophy, wheelchair bound, and fed through a percutaneous gastrostomy. Finally, a girl from the extended family (the two fathers are brothers) was also noted from birth with hypoglycemia (0.39 mmol/L) (Figure 69.3). Blood glucose stabilized quickly after glucose infusion. Hyperbilirubinemia (total bilirubin 345.3 μmol/L, direct bilirubin 6.8 μmol/L) at day two required intensive

Figure 69.2 A boy with ADK deficiency. He had a hypotonic-dystonic movement disorder. Epilepsy began at the age of four months. Frontal bossing had been noted. Communicated with sounds and gesticulation. He was nearly blind, as a result of retinal dystrophy.

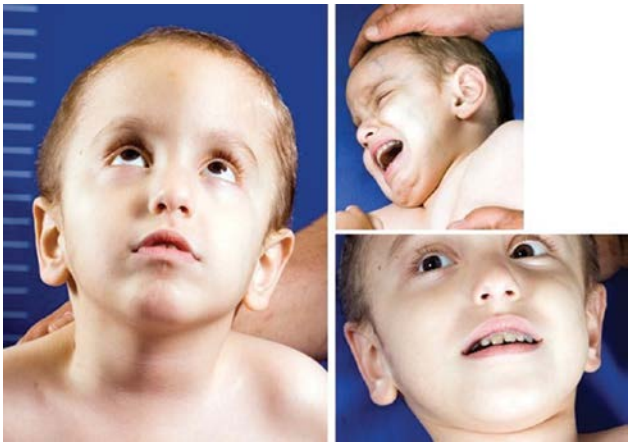

Figure 69.3 A four-year-old girl with ADK deficiency. She had frontal bossing, severe neurologic impairment with epilepsy and pronounced psychomotor retardation. Yellowish-greenish teeth were attributed to hypermethioninemia and hyperbilirubinemia. Cholestatic liver disease was most severe in her first year of life, when she developed liver failure. Hepatopathy ameliorated under a low-methionine diet. At the age of 14 years, a malignant, hepatocellular carcinoma was diagnosed.

phototherapy. Newborn screening from dried blood spot (DBS) revealed hypermethioninemia (138 μmol/L, N < 91; day four of life), but on day 11, methionine had become normal (70 μmol/L). Over the next months, liver disease worsened with cholestatic hyperbilirubinemia and hepatomegaly progressing to liver failure and hepatorenal syndrome with metabolic acidosis and ascites. Massive hypermethioninemia (methionine 809 μmol/L) with clearly elevated AdoMet (2299 nmol/L), and moderately increased AdoHcy (187 nmol/L) in plasma was now obvious. In a desperate situation, a low methionine diet was started, and the family decided to take her daughter home but to continue the low methionine diet in a constellation which was believed to be palliative. However, liver function slowly stabilized and improved until about one year later, it became normal. At the age of two years, the diet was stopped but successfully reintroduced one month later, as impairment of liver function and hypermethioninemia recurred. There was severe neurologic impairment with epilepsy and pronounced psychomotor retardation. At the age of 14 years, she had been diagnosed with a multifocal intra- and extrahepatic tumor, with high levels of alpha-fetoprotein and histologic signs of hepatocellular carcinoma. These three cousins shared the homozygous missense mutation (c.905 T > C, p.Phe302Ser) in the *ADK* gene.

By now in 20 reported patients [1–3], developmental delay and dysmorphic features with frontal bossing have uniformly been present from or shortly after the first months of life, whereas hypertelorism, sparse and thin hair, abnormal dentition, Marfanoid features such as slender hands and feet, macrocephaly are inconstant findings. Muscular hypotonia may be noted at birth. About 70 percent of patients develop epilepsy with a mean age at onset of two years which is often difficult to treat. Intellectual disability ranges from moderate to severe. Two of the oldest patients, 36 and 30 years old, had a severe neurologic phenotype with pronounced mental disability, very severe behavioral abnormalities, and refractory epilepsy; whereas two other siblings, aged 29 and 35 years, had moderate mental impairment, could walk without any imbalance, and do their personal work without help.

First symptoms can be severe or prolonged conjugated hyperbilirubinemia and hypoglycemia. Liver disease is variable with hepatomegaly, increases of liver transaminases and cholestasis, ranging from mild hepatopathy to liver failure. It tends to improve with increasing age. Microvesicular hepatic steatosis is a typical finding. Furthermore, in five patients examined, liver biopsy revealed portal or periportal fibrosis (four patients), cholestasis (two patients), a reduced number of bile ducts (two patients) and mild lobular hepatitis (one patient) [2].

Systematic evaluation of 21 MRIs from eight patients (age range nine days – 14.6 years, mean 3.9 years, median 2.7 years) failed to demonstrate specific abnormalities [4]. Brain maturation was delayed, but ultimately completed often with nonspecific white matter changes, and potentially transient central tegmental tract hyperintensity (Figure 69.4). As creatine synthesis depends on methylation from AdoMet utilizing about 40 percent of whole body AdoMet [5], cerebral creatine deficiency could have been anticipated in ADK deficiency. However, no indication of low creatine was found on ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) in five patients [4].

ADK converts adenosine to AMP, and defective function of the enzyme leads to an accumulation of adenosine. Adenosine is elevated in dried blood spots from affected neonates and could be potentially used as a biomarker in newborn screening [2]. However, in some patients examined after the neonatal age, adenosine concentrations in urine were only slightly elevated or in the upper normal range and can show great variation within the same individual. Laboratory evaluation mostly reveals hypermethioninemia, up to 1100 μmol/L [1, 2]. However, methionine levels vary and may even lie within the normal range. Measurements of AdoMet and AdoHcy in plasma appear to be more sensitive and specific than methionine, but are only performed in very few specialized metabolic laboratories. AdoMet has been elevated at all times in all known patients. AdoHcy has been normal in one of the patients described by Bjursell but was elevated in all others [1, 2]. Elevations of plasma AdoMet were typically ~2-20-fold (up to 2299 nmol/L, n 71-118), while AdoHcy was ~5-30-fold elevated (up to 438 nmol/L, n 9.3-14). Total homocysteine can be mildly elevated. CSF levels of methionine and the two adenosyl compounds are also elevated. Activities of SAHH in fibroblasts of the two Swedish siblings were found to be normal.

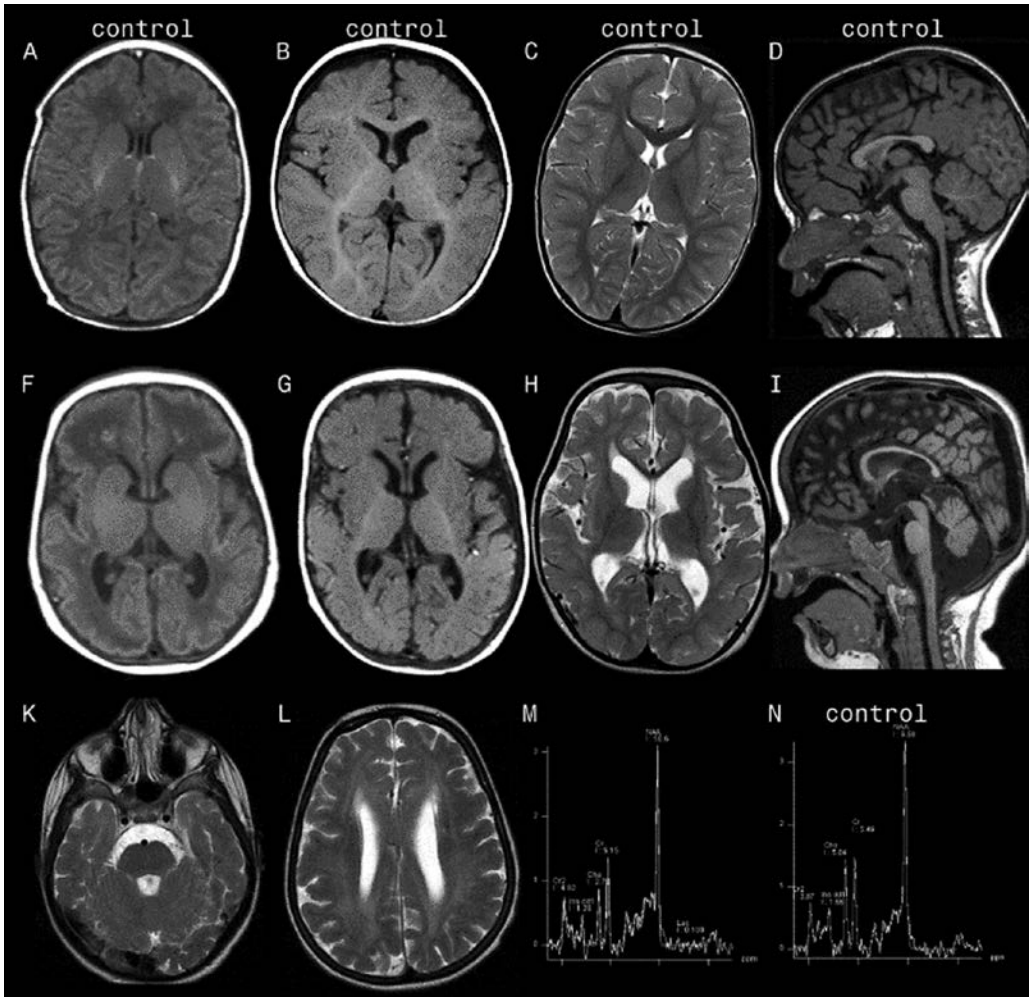

Figure 69.4 MRI and MRS changes in ADK deficiency. A–I: MRI pictures of patients at 12 days (F), 6.8 months (G), and 2.7 years (H, I) compared to control images from a term neonate at day ten (A), a 6.5-month-old child (B), and from a 2.7-year-old child (C, D). F: Immature gyral pattern and white matter signal at day 12 with absent myelination of the pyramidal tract in the posterior limb of the internal capsule (compare with A). Additional findings are subependymal cysts and punctate white matter T1-hyperintensities (F). G: At 6.8 months, the gyral pattern was mature; myelination had progressed, but was still delayed; cysts and punctate hemorrhages were resolving. The ventricles were wide (compare with B). H, I: Mild periventricular white matter changes, wide lateral ventricles and a thin corpus callosum at 2.7 years (compare with C, D). K–M: Different patients with ADK deficiency displaying slight central tegmental tract hyperintensity (K), diffuse supratentorial white matter changes sparing the optic radiation and corona radiata (L), and visibly lower choline resonances (M) compared to a control spectrum. (N); Cho/Cr was 4 SD below mean of controls. Creatine was visually normal. (This figure was kindly provided by Dr Inga Harting of the Department of Neuroradiology, University Hospital Heidelberg, Germany.)

GENETICS AND PATHOGENESIS

Exome sequencing of the Swedish siblings identified the homozygous missense mutation c.902C > A (p.Ala301Glu) in the *ADK* gene in exon 10 [1]. In the Malaysian patients, a c.653A > C transversion causing p.Asp218Ala was found in one Malaysian set of siblings and c.38G > A (p.Gly13Glu) in the other. Staufner and colleagues described seven new mutations of the *ADK* gene in eleven patients from eight families, including missense and nonsense mutations, a deletion, and a frameshift mutation [2].

Methionine is an essential amino acid that is not metabolized by transamination as are other amino acids. The methionine cycle includes adenosylation of methionine (see Figure 69.1) catalyzed by methionine adenosyltransferase (MAT I/III) to AdoMet, donation of the methyl group forming AdoHcy via various methyltransferases, and hydrolysis by SAHH to generate homocysteine and adenosine.

ADK converts adenosine to AMP. A defective function of the enzyme leads to an accumulation of adenosine and reverses the reaction balance of SAHH, causing elevated

concentrations of AdoHcy, AdoMet, and methionine, which are the biochemical hallmarks of the disease (Figure 69.1) [1, 2]. Interference with a wide range of methyltransferases was deemed [1] likely.

An Adk knockout mouse had progressive fatal microvesicular hepatic steatosis. Reduced concentration of adenine nucleotides including ATP depletion in liver homogenates was discussed to impair mitochondrial lipid metabolism, contributing to microvesicular steatosis [6]. Along with citrin deficiency (Chapter 28), Niemann-Pick Type C (Chapter 91) or mannosephosphate isomerase deficiency, ADK deficiency should be included in the metabolic differential diagnosis of transient infantile cholestasis.

The differential diagnosis of hypermethioninemia includes, in addition to ADK deficiency, deficiencies of methionine adenosyltransferase (MAT I/III), glycine N-Methyltransferase (GNMT), S-adenosylhomocysteine hydrolase (SAHH), and cystathionine β-synthase (CBS) (Chapter 18). Elevated methionine concentrations are also commonly present in hepatic disease and in hepatorenal tyrosinemia and citrullinemia type 2 as well as other nonmetabolic liver diseases, but then hypermethioninemia is rarely found in isolation but rather in combination with elevated tyrosine and phenylalanine.

Inhibitors of ADK were shown to specifically promote replication of primary β-cells of the pancreas *in vitro* and *in vivo* through activation of the mTOR pathway in the animal model [7]. After replicating this finding, another group suggested that adenosine signalling has several independent mechanisms promoting ß-cell proliferation. They demonstrated the involvement of adenosine receptor A2aa in an intracellular increase of cAMP through the G protein coupled adenylate cyclase [8], providing an additional explanation for hyperinsulinism in ADK deficiency.

TREATMENT

No established treatment is known. A methionine restricted diet has been recommended [2] and has been applied in the siblings published by Labrune in 1990 [3]. Taken together, ten patients were prescribed a methionine restriction of 15–40 mg/kg body weight/day. Methionine levels decreased, as did those of plasma AdoMet and AdoHcy. Seven patients were considered to have improved clinically, especially in their hepatic features. In one, hepatic dysfunction returned when the diet was stopped even at the age of 20 years and reversed on resumption. One patient showed improvement of his neurologic phenotype with amelioration of cognitive and motor deficits (Figure 69.5). Diazoxide may be used to treat hyperinsulinism [2].

Figure 69.5 Another patient with ADK deficiency at the age of four (left) respectively five years (right). He had a milder phenotype, but frontal bossing was still obvious. At the age of 2.5 years, he had feeding difficulties, recurrent vomiting, hypoglycemia, increased transaminases, neurodevelopmental delay, and ataxia. A low methionine diet was started and feeding difficulties improved strikingly, with disappearance of vomiting, no further need of tube feeding and almost complete normalization of hypoglycemia and liver function. Improvement of developmental delay and speech was also observed, and the patient achieved autonomous gait. (Illustrations of this patient were kindly provided by Dr Carlo Dionisi-Vici of the Bambino Gesù Children's Research Hospital IRCCS in Rome, Italy.)

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70

Purine nucleoside phosphorylase deficiency

MAJOR PHENOTYPIC EXPRESSION

Severe immunodeficiency in which T-cell function and B-cell function are abnormal. The severe combined immunodeficiency (SCID) may be clinically indistinguishable from adenosine deaminase deficiency; lymphopenia, thymic deficiency and infections; hypouricemia; and deficient activity of purine nucleoside phosphorylase.

INTRODUCTION

A patient with deficiency of purine nucleoside phosphorylase (PNP) (Figure 70.1) was reported in 1975 by Giblett and colleagues [1]. The girl was a five-year-old with severe deficiency of T-lymphocyte-mediated immunity. She had had a long series of infections and was found to have marked lymphopenia. Skin tests for delayed hypersensitivity were negative, and her lymphocytes failed to respond to phytohemagglutinin. On the other hand, B-lymphocyte-medicated immunity was normal, as indicated by normal levels of IgA and IgM, an elevated level of IgG and normal antibody responses to immunization. There was a small number of other patients reported [2–6], in each of whom there was selective severe T-cell deficiency. It is now clear that some patients with PNP deficiency may have abnormal B-cell function as well [7, 8]. In these patients, levels of immunoglobulins are reduced.

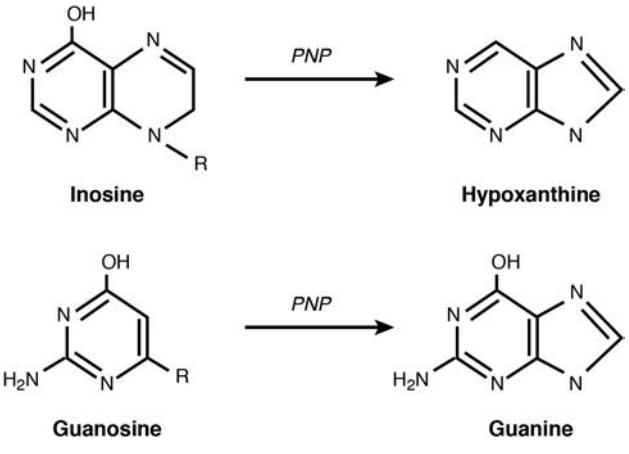

Figure 70.1 The purine nucleoside phosphorylase (PNP) reaction, site of the defect. The enzyme also catalyzes the conversion of deoxyinosine and deoxyguanosine to hypoxanthine and guanine.

Deficiency of PNP is unique among immunodeficiencies in that patients have impressive hypouricemia [9, 10] and a very low level of excretion of uric acid in the urine. These features provide a diagnostic marker for the disease [11]. Concentrations of inosine and guanosine in the serum are markedly elevated, as is their excretion in the urine [3, 12]. These patients overproduce purines de novo, which appears to be a consequence of the accumulation in cells of PRPP [6]. These observations suggest a role for purine nucleoside phosphorylase in providing a source of hypoxanthine for nucleotide synthesis catalyzed by hypoxanthine guanine phosphoribosyl transferase (HPRT). In the absence of substrate hypoxanthine or in the absence of HPRT, levels of PRPP rise.

The *PNP* gene was localized to chromosome 14qll.2 [13]. Compound heterozygosity for the two mutations p.D128G and p.R234P was reported in a patient with deficiency of the enzyme [8]. Another patient with compound mutations was reported [14]. Twenty-four disease causing mutations have been reported [15].

CLINICAL ABNORMALITIES

Serious viral infections have been encountered in these patients. One developed vaccinia gangrenosa following vaccination against smallpox [2]. Postmortem examination revealed a hypoplastic thymus with marked cortical depletion and hypoplasia of T-cell generated areas of lymphoid tissues. The development of impaired T-cell function was documented in a patient [3] in whom T- and B-cell immunity were normal at birth, after which T-cell function gradually decreased until she was admitted to hospital with otitis media at 15 months. At that time no tonsils, lymph nodes or thymus could be documented clinically. The bone marrow was megaloblastic and the blood hypochromic and microcytic. In this family, two immunodeficient siblings had died of a lymphosarcoma and a graft versus host reaction following a blood transfusion. The patient had a slight spastic tetraparesis and slight retardation of mental development. This was also present in her eldest involved sister. Another patient was well until she developed otitis media at 18 months [4]. Thereafter, she carried a chronic infection with cytomegalovirus and developed an autoimmune hemolytic anemia. There were hardly any E-rosetteing T-cells and T-cells were some 10 percent as tested with anti-T-cell serum. Skin tests with candida and streptokinase/steptodornase, as well as PPD were streptokinase/steptodornase negative. B-cell function was initially normal, but there was progressive loss of B-cell function. She died of interstitial pneumonia, and there was evidence at autopsy of extensive involvement of both B- and T-cell systems.

Autoimmune hemolytic anemia was also present in a boy who presented first at three years of age with an influenza-like illness and extreme lymphopenia in which there were 282 lymphocytes per cmm [5]. In this patient, B-cell function was normal. The T-cell mediated *in vitro* response to phytohemagglutinin was markedly depressed.

Neurologic abnormalities have been reported in about half the patients [5, 16, 17]. Varying degrees of developmental delay have been associated with spastic tetraparesis. Patients have manifested hypotonia or hypertonia and tremor. Actually, Hagberg *et al.* [18] reported a brother and sister with ataxic diplegia and defective cellular immunity five years prior to Gilbert's report [1]. The sister had vaccinia gangrenosa at 15 months which was successfully treated. She died at four years of age of generalized varciella. The brother died of a cerebral abscess at five years. Another two-year-old boy with PNP deficiency had spastic tetraplegia and died of a malignant B-cell lymphoma [19]. A three-year-old who presented with spastic diplegia and a behavior disorder did not develop manifestations of immunodeficiency until five years of age [20]. Familial disequilibrium and pyramidal tract signs without prominent spasticity were reported along with defective cell-mediated immunity and death from lymphoma. Mild developmental delay was observed [14] in a boy who had uneventful varcella at eight months, but then a prolonged parvovirus infection at 26 months. He had ataxia, decreased T-cell number and mitogenic response.

GENETICS AND PATHOGENESIS

Nucleoside phosphorylase deficiency is an autosomal recessive disease. Parental consanguinity had been reported [4].

PNP enzyme activity is often undetectable [3, 4, 14]. Activity in heterozygotes has been reported at about half of control values [14].

Accumulated PRPP in turn drives the de novo pathway of purine synthesis. In one patient, physicochemical evidence of structural gene mutation was found in an increased Km for inosine and decreased heat stability [12]. Because no activity at all could be detected in the propositus, these studies were carried out in heterozygotes in which the six electrophoretically separate bands seen in normals were present. The normal human enzyme has a trimeric structure [21]. It has been postulated [3] that in the patient there is a catalytically inactive trimer.

A normal electrophoretic pattern was obtained in another family [22], but molecular heterogeneity has been demonstrated by the fact that starch gel electrophoresis of the enzyme of two patients in one family [22] disclosed a reduced number of isozymes, and the parents in two families had additional slower moving bands [1]. A patient with severe T-cell dysfunction and normal B-cell function was found to have no erythrocyte PNP activity and no immunoreactive material [23].

PNP (see Figure 70.1) catalyzes the reversible conversion of inosine to hypoxanthine, guanosine to guanine, and also xanthosine to xanthine. Activity of the enzyme is readily assayed in erythrocytes, and patients have been reported to have no detectable activity [3]. The defect is also demonstrable in lymphocytes [12] and in granulocytes [4].

Recently described novel mutations in the gene for PNP include c.710A > G which resulted in p.H237R and c.237A > G which led to p.H86R in two siblings and c.199C.T leading to p.R67X [15]. Among seven mutations recently summarized [15], five led to a clinical phenotype in which plasma uric acid was normal. Thus, it has been advised that a diagnosis of deficiency of PNP should not be excluded in the absence of hypouricemia. Another source of confusion was illustrated [15] by a patient in whom activity of the PNP enzyme resulted from multiple transfusions of blood. A child with deficiency of PNP, an Arabian product of first-cousin mating, also had normal levels of uric acid [24]. Two older sisters had died of recurrent infections. The mutation was c.487T > C in the PNP gene resulting in the substitution of proline for serine at amino acid residue 163.

The abnormality in purine metabolism in purine nucleoside phosphorylase deficiency resembles that of adenosine deaminase deficiency in that each leads to the

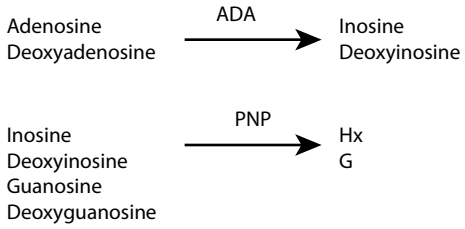

Figure 70.2 Defects in purine metabolism associated with Immunodeficiencies.

accumulation of nucleosides and deoxynucleosides (Figure 70.2). These are rapidly converted to nucleotides and the major increases in purine products observed are the trinucleotides. Thus, as in adenosine deaminase deficiency dATP accumulates, in PNP deficiency dGTP accumulates [25, 26]. Deoxyguanosine, like deoxyadenosine, kills cultured T lymphoblasts, but B lymphoblasts are relatively resistant to deoxyguanosine [27–29]. The association of the toxicity of deoxyguanosine and the accumulation of high concentrations of dGTP is thought to result from the fact that dGTP is a potent inhibitor of ribonucleotide reductase [30–32]. This is thought to inhibit the synthesis of DNA [33]. Disordered immune function in these conditions is considered to result from the death of immunocompetent cells.

Neurologic dysfunction in this disease has been attributed to deficiency of the products of the PNP reaction see (Figure 70.3) hypoxanthine and guanine. These are, of course, the subjects of the hypoxanthine guanine phosphoribosyltransferase (HPRT) reaction. In this sense, there are some similarities to the neurology of Lesch-Nyhan disease, but some notable differences include the abnormalities in behavior in HPRT deficiency.

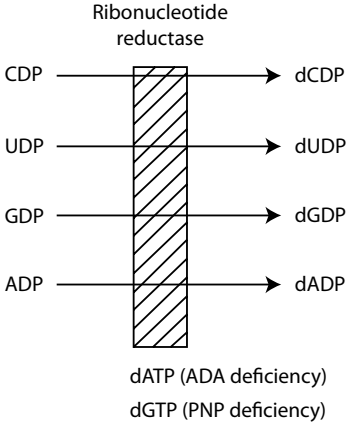

Figure 70.3 Ribonucleotide reductase. It is thought that the metabolic abnormalities in adenosine deaminase (ADA) deficiency and purine nucleoside phosphorylase (PNP) deficiency, in which the deoxynucleotides of adenine (dATP) and guanine (dGTP) accumulate lead to the inhibition of ribonucleotide reductase and thereby interfere with DNA synthesis.

TREATMENT

Definitive treatment can be achieved by means of bone marrow transplantation. Transfusion therapy was developed in the management of patients with adenosine deaminase deficiency [34]. In PNP, deficiency transfusion therapy has variously been reported to produce partial improvement or no improvement in immune function [35–38]. In an extensive experience with 100 weeks of erythrocyte transfusion therapy in a boy with PNP deficiency, there was a correction of the elevated level of dGTP in erythrocytes and leukocytes, as well as a substantial increase in serum concentrations of urate and decrease in urinary nucleoside content [7]. The immunologic abnormality was partially reversed. However, the overall results of therapy in this disease have been much less effective than in adenosine deaminase deficiency [39].

Protein transduction domain (PTD) fusion products with PNP, utilizing the PTD from the TAT protein from the human immunodeficiency virus (HIV) have been used to facilitate transfer of enzyme molecules across cell membranes [39]. This approach has been shown to correct PNP deficiency in lymphocytes derived from a patient with PNP deficiency. Abnormalities in immune function in PNP -/- mice were corrected by incubation of T lymphocytes with PTD-PNP.

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71

Adenylosuccinate lyase deficiency

MAJOR PHENOTYPIC EXPRESSION

Developmental impairment, seizures, autistic behavior, excretion of adenylosuccinate and succinylaminoimidazolecarboxamide riboside in the urine and deficient activity of adenylosuccinate lyase.

INTRODUCTION

Adenylosuccinate lyase (ASL) deficiency was first described by Jaeken and Van den Berghe [1] in 1984. This created enormous interest because autistic behavior was observed in the affected patients in this family, and it would be of considerable interest if it were possible to relate molecular changes in the gene for ASL to the genetics of autism [2]. However, extensive survey of autistic populations has failed to turn up additional patients with lyase deficiency. A more typical phenotype includes seizures and developmental delay [3].

The enzyme adenylosuccinate lyase (adenylosuccinase, ASL; EC 4.3.2.2) catalyzes the eighth step in the *de novo* synthesis of purines in which succinylaminoimidazolecarboxamide ribotide (SAICAR, SAICAMP) is converted to aminoimidazolecarboxamide ribotide (AICAR, AICAMP, ZMP) (Figure 71.1) [4, 5]. The same enzyme catalyzes the conversion of adenylosuccinate to adenosine monophosphate (AMP) in the cycle of purine nucleotide conversions that yield adenine nucleotides [6]. Deficient activity of the enzyme was documented in 1988 by Jaeken and colleagues [4]. The human gene has been mapped to chromosome 22q1.3.1.-1.3.2 [7]. The human cDNA has been cloned, and

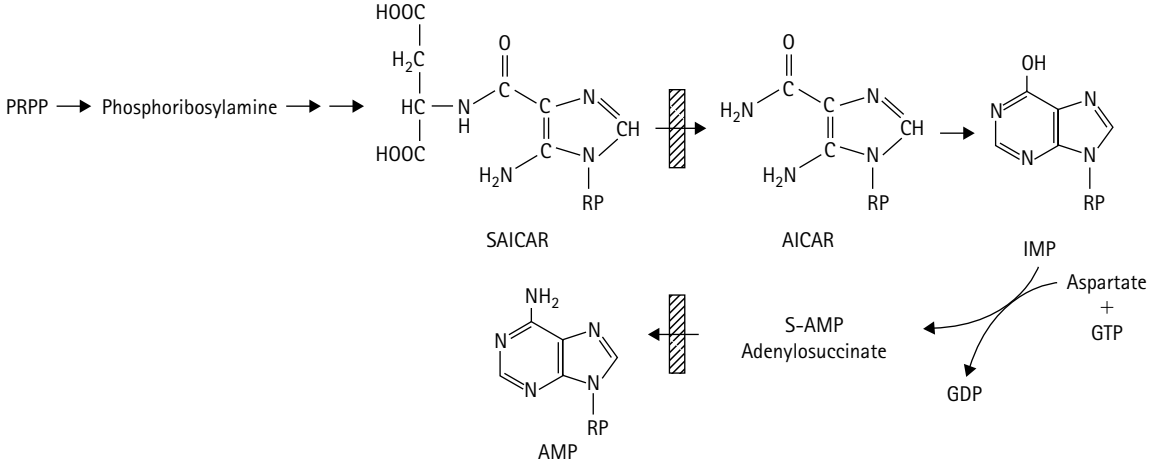

Figure 71.1 The reactions catalyzed by adenylosuccinate lyase (ASL). In the *de novo* pathway of purine nucleotide synthesis the conversion of 5-phosphoribosyl-5-amino-4-imidazole succinyl-carboxamide (SAICAR) to 5-phosphoribosyl-5-amino-4-imidazole carboxamide (AICAR) and in the purine interrelations cycle the conversion of adenylosuccinate to adenylic acid (AMP).

the nature of the point mutation was defined in the initial family reported [1, 2]. A majority of the 30 different mutations initially delineated were missense, most of them in compound heterozygotes. The most common mutation, p.R426H, was found in 17 families from many countries [3, 8] many on single alleles. More than 50 patients have now been reported [3]. Mutations have continued to be missense, many on single alleles.

CLINICAL ABNORMALITIES

The phenotype of ASL deficiency is variable, but it is clear that psychomotor impairment is a regular manifestation of the disease [1, 4, 6, 9, 10]. Many have had seizure disorders. Some of the patients with early onset seizures have died in infancy [11–15]. More have had moderate to severely impaired mental development and seizures after the first year [1, 4, 9]. One patient experienced a fatal neonatal disease [3]. The infant died of respiratory failure having shown no spontaneous movements. Autistic features in some have included absence of eye contact, repetitive behavior, temper tantrums, and self-injurious behavior, none of them rare in individuals with impaired mental development. Impaired growth and muscle wasting have also been observed [4]. Three patients had only mild developmental delay [3, 4]. They have been referred to as type II to distinguish them from all the other patients in type I, in whom impaired mental development is more severe [16]. Another patient was described [9] as having an intermediate degree of symptomatology and another had only delayed motor development and severe hypotonia [17]. Siblings shown in Figure 71.2 had less severely impaired mental development [11]. It seems likely that once a large number of patients is observed, a spectrum will be the case rather than discrete groups of phenotypes [18].

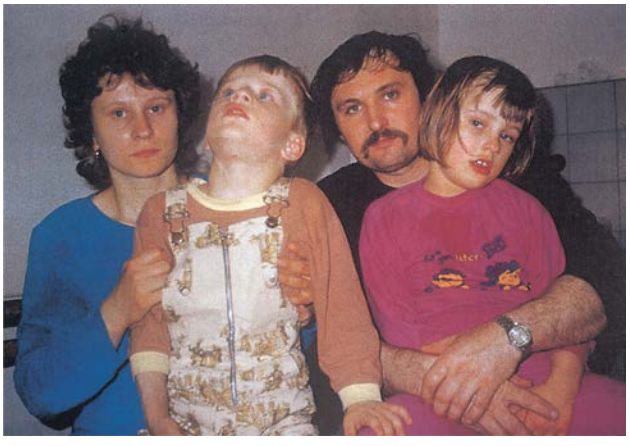

Figure 71.2 Two siblings with ASL deficiency, shown with their parents. Their degree of mental impairment was described as less severe. (This illustration was kindly provided by Dr Ivan Sebesta of Universita Karlova, Prague, Czech Republic.)

Facial dysmorphic features were reported [19] in two patients who had brachycephaly, a short nose with anteverted nares, a smooth philtrum, thin upper lip, brachycephaly, and low set ears.

A behavioral phenotype has been reported [20] resembling Angelman syndrome. Two sisters, 11 and 12 years of age, had global developmental delay, motor apraxia, and severe deficits in speech. They also had seizures. The behavior was characterized by happy dispositions and excessive laughter. They were hyperactive and had short attention spans. They mouthed objects and had tantrums and stereotypical movements. Self-injurious behavior has been observed in this disease [1, 3, 21].

A distinctive feature of ASL deficiency that simplifies the detection of this disorder is the accumulation of the metabolites adenylosuccinate (succinyladenosine) and SAICAriboside (succinyl-AICAriboside), the dephosphorylated products of the substrates for the deficient enzyme. It is possible to screen for the latter compound, because it gives a positive Bratton-Marshall reaction (Figure 71.3) [22]. Confirmation of a positive screening test is done by identification of adenylosuccinate and SAICAriboside in urine or blood [1]. Both compounds are also readily found in the cerebrospinal fluid (CSF), where concentrations are 20- to 100-fold those of plasma and are as high as 500 mmol/L [1, 23]. Urinary excretions range from 25 to 700 mmol/mol creatinine [1, 4, 23]. In most patients with the classic neonatal phenotype, the ratio of the two compounds adenylosuccinate/succinyl-AICAriboside in the CSF approximates 1 [3, 10]. Patients with milder phenotypes have had more adenylosuccinate, sometimes as much as four-fold higher or even 100-fold [4], and the ratio is over 2. In the severe neonatal disease, the ratio is less than 1.

The diagnosis has been made via untargeted metabolomic profiling, and confirmed by targeted quantitative biochemical analysis. Screening for the disease has been performed via tandem mass spectrometry for succinylpurines in dried spots of blood [24].

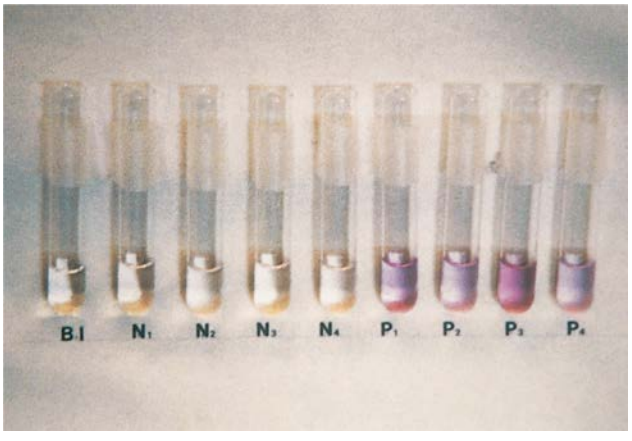

Figure 71.3 The Bratton-Marshall reaction. On the left are negative tubes; on the right, the urine of two patients with ASL deficiency.

GENETICS AND PATHOGENESIS

The disorder is inherited as an autosomal recessive trait. Consanguinity has been described [1]. The enzyme has been generally assayed by following the conversion of adenylosuccinate to AMP spectrophotometrically [25]. Liver, fibroblasts, and lymphocytes have been used to document the deficiency of enzyme activity in individuals with ASL deficiency [4, 10, 26]. The very different metabolite ratio in the type II patient suggested that the enzyme be assayed with both substrates. It was found that in classic type I ASL deficiency, the activities of the enzyme toward the two substrates are decreased in parallel to about 30 percent of control [9]. In general, the activity of mutant enzymes against both substrates are proportionately decreased, regardless of clinical phenotype [27]. In one study of fibroblasts derived from a patient with a type II genotype, activity against succinylAICAR was about 30 percent of control, but when adenylosuccinate was the substrate, the activity was only 3 percent of control [10], data consistent with the higher concentration of adenylosuccinate in the type II patient.

Kinetic studies have indicated that in lymphoblasts [28], as well as in fibroblasts [10], the affinity for adenylosuccinate is normal even at physiological temperatures [2]. Furthermore, the variant enzyme has been shown to have decreased stability to heat [28], and this would be consistent with the reduced residual activity. An increase in the Km for succinylAICAR in some patients [10] indicates modification of the active site. The kinetics of the type II enzyme differed in that the Km for adenosylsuccinate was markedly increased [10]; its V_{max} was strongly inhibited by KCl and nucleoside triphosphates, neither of which affected the kinetics of type I. Mammalian ASL is a heteropolymer of about 52 kDa containing four subunits [29].

The cDNA for the gene contains 1452 nucleotides and codes for a protein of 484 amino acids [27]. Molecular analysis in the first reported Moroccan family with four affected children [1, 2, 4] indicated homozygosity for a point mutation in the *ASL* gene resulting in a serine to proline change originally placed at amino acid 413, but now called S438P in the 484 amino acid protein. This might be expected to increase the flexibility of the peptide backbone of the enzyme, which might account for decreased stability. Analysis of genomic polymerase chain reaction (PCR) products from the parents of the patients revealed both a normal and a mutant allele, documenting heterozygosity [2]. The mutation p.R426A remains the most common mutation in a variety of unrelated patients [3]. Another, p.Y114H, has also been found in more than one unrelated family [3]. Most mutations continue to be private. Another mutation identified [30] in a gypsy patient without known consanguinity, G1279A, converted a well-conserved arginine at 401 to histidine. Other missense mutations identified have indicated a high degree of molecular heterogeneity [30–32]. A 39-bp deletion in the cDNA was caused by a C to A change in exon 5 creating a consensus 59 donor splice site [33]. One nonsense mutation has been observed [27].

An interesting mutation was found in three unrelated patients [8]. The coding sequence was normal in the allele with the mutation, which was a c.49T > C change in the 5'-untranslated region (UTR). This led to a reduction to about 25 percent of wild-type promoter function and mRNA. The mutation affected the binding of a known activator of transcription, nuclear regulatory factor 2 (NRF-2). These observations are consistent with a role for NRF-2 in the regulation of purine synthesis. In a patient with autistic features, two more mutations E80D and D87E were found [34]. In a study of R420H and R303C [35] evidence was found of non-linear dependence of the activities on the ratio of the two substrates resulting from competitive binding to the differing enzyme substrate [35].

Despite the continuing discovery of novel mutations [34], correlations between genotype and phenotype have been elusive.

TREATMENT

Specific therapy has not been devised [36]. Seizures may be treated with the usual anticonvulsant drugs. Management is designed for optimal developmental potential. A 12-month trial of ribose therapy was without effect [3, 37, 38].

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72

Xanthinuria, xanthine oxidase deficiency

MAJOR PHENOTYPIC EXPRESSION

Urinary tract stone disease with xanthine calculi, hematuria, urinary tract colic and infection, muscular cramps, hyperuricemia, xanthinuria, and deficiency of xanthine oxidase.

INTRODUCTION

Xanthinuria is a rare inborn error of purine metabolism in which the activity of xanthine oxidase is deficient in tissues. Individuals may be asymptomatic for many years. They may be discovered through a routine determination of the concentration of uric acid in the blood, which is unexpectedly low. A number of the patients reported have had xanthine calculi in the urinary tract. A few patients reportedly have had painful cramps in muscles and deposits of xanthine crystals in muscle.

Xanthinuria was first definitively reported by Dent and Philpot [1] in 1954 in a four-year-old girl who had hematuria and urinary frequency and passed a smooth oval, radiolucent stone which was found to be composed of xanthine. This same patient was later studied at 9, 14, and 19 years of age [2] and found to have some persistent clubbing of the left renal calyces and reduced size of the left kidney but to be normotensive and in good health.

The molecular defect in xanthinuria is in the enzyme xanthine oxidase (Figure 72.1). This enzyme catalyzes the conversion of hypoxanthine to xanthine and of xanthine to

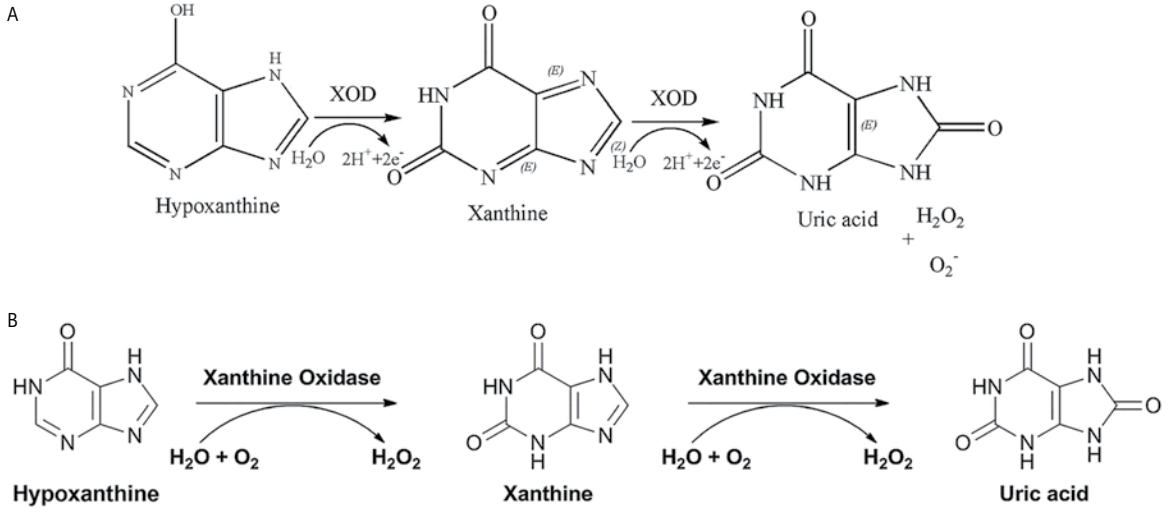

Figure 72.1 The xanthine oxidase reactions. Both conversions are catalyzed by the same enzyme.

uric acid. The enzyme is found normally in liver, intestinal mucosa, and milk. The activity of xanthine oxidase has been found to be deficient in biopsied liver and jejunal mucosa in patients with xanthinuria [3–5].

In three individuals with xanthinuria Type I there was a C-to-T transition at nucleotide 682 of the dehydrogenase gene was found to be homozygous [6]. A deletion of C at nucleotide 2367, coding for a stop was also homozygous. In a fourth, it was present with deletion of C at nucleotide 2367 coding for a stop.

Xanthinuria type II has been observed in conjunction with deficiency of aldehyde oxidase. The mutation has not been reported. Type I individuals metabolize allopurinol. Type II patients cannot.

Xanthinuria also occurs in molybdenum cofactor deficiency (Chapter 74).

CLINICAL ABNORMALITIES

Any patient with urinary tract calculi may develop urinary tract colic, hematuria, acute obstruction, or urinary tract infection. Xanthine stones, like those of uric acid or cystine, are radiolucent, and thus contrast roentgenographic study or ultrasound is usually required for their visualization. Occasionally, co-precipitation of calcium salts in the calculus will permit visualization on a plain roentgenogram. In most reported patients, even with stones, the course has been relatively benign, but severe recurrent stone disease has been observed, leading to hydronephrosis and a nephrectomy at 12 years of age [7]. Many individuals with xanthine oxidase deficiency have avoided stone formation, even in advanced ages. However, a seven-month-old girl with urolithiasis has been reported [8].

An unusual presentation has been reported in two patients in whom myopathy was associated with crystalline deposits of xanthine in skeletal muscles [3, 4, 9]. One of these patients a 23-year-old black patient with pheochromocytoma, mental retardation, glucose-6-phosphate dehydrogenase deficiency, and congenital skeletal anomalies [4] developed cramps in her muscles after walking, and was found to have crystals in biopsied muscle which were identified as oxypurines [10, 11]. The other patient, a 31-year-old black patient from Guyana, who had been an active athlete until three years previously, developed tight or distended sensations in his thighs and calves. The calves felt abnormally firm to examination, and the electromyogram revealed a myopathic pattern, Muscle fibers contained rod-like crystalline inclusions which were identified as oxypurines [10, 11].

The diagnosis of xanthinuria is strongly suggested by a concentration of uric acid in the serum that is less than 1 mg/dl. Hypouricemia is also seen in patients with a variety of renal tubular abnormalities, including the Fanconi syndrome with or without cystinosis, Wilson disease, and a primary defect in the tubular absorption of urate.

Table 72.1 Solubility of Oxypurines in Urine

	pH5 mg/dl	pH7 mg/dl
Uric Acid	15	200
Hypoxanthine	140	150
Xanthine	5	13

Note: The data were reported by Klinenberg *et al.* [15].

Plasma levels of xanthine are also elevated in xanthinuria, but this determination is difficult to do properly because of rapid movement of oxypurines out of erythrocytes into the plasma. The diagnosis is made more easily by examining the urine for the oxypurines, xanthine, hypoxanthine, and uric acid [12]. In a normal adult, the 24-hour urinary excretion of xanthine approximates 5 mg and that of hypoxanthine 10 mg, while that of uric acid is up to 500 mg. In xanthinuria, the excretion of uric acid is generally less than 30 mg and the approximate 500 mg of purine normally excreted as uric acid is found 85 percent in xanthine and 15 percent in hypoxanthine. An average excretion of xanthine while the subject was ingesting a standard low purine diet was 0.26 mg/mg of creatinine [13]. Total oxypurine excretion of 0.5 to 0.6 mg/mg of creatinine has been reported [14]. That of hypoxanthine was 0.05 mg/mg of creatinine. The amounts of oxypurine being formed are not large. They are simply being excreted largely as xanthine. The size of the xanthine pool in such a patient is similar to that of hypoxanthine, and its turnover is about one-third that of hypoxanthine, but approximately 80 percent of the xanthine turned over daily appears in the urine, while only 5 percent of that of hypoxanthine appears in the urine. Thus, there is a major reutilization of hypoxanthine and little of xanthine. These metabolic features are important for the patient because of the insolubility of xanthine (Table 72.1). By contrast with xanthine, uric acid is relatively soluble in human urine at pH values that are achievable. For these reasons in the presence of xanthinuria the formation of calculi is to be expected.

Allopurinol is effective in the treatment of gout and myeloproliferative disease by virtue of its effectiveness as an inhibitor of xanthine oxidase [15].

GENETICS AND PATHOGENESIS

Xanthinuria is an autosomal recessive disorder. It has been reported in two brothers whose parents were normal [7]. A reliable method of heterozygote detection has not been reported.

Enzyme assay requires biopsy of the liver or intestinal mucosa, so it is not frequently evaluated. Reported activity has ranged from undetectable to less than 10 percent of control [3–5, 16–18].

The gene for xanthine dehydrogenase is located at chromosome 2p22.3-22.2. Patients with reported mutations

(v.i) had no dehydrogenase activity [19] in biopsied intestinal mucosa, but levels of mRNA were not reduced. In another Japanese man found to have hypouricemia in a health check-up, a 445C > T transition was found in exon 6 [20]. A mutation in exon 25, g.6477C > T (c.2808 C > T, p.T910M) was reported [21]. This mutation was found along with a mutation in exon 8, g.2707delC (c.720delC, p.214Qfsx) [22].

Patients with the common Type I xanthinuria cannot metabolize allopurinol to oxypurinol. They also could not metabolize pyrazinamide [23] to 5-hydroxypyrazinamide. Xanthinuric patients unable to convert allopurinol to oxypurinol also had deficient activity of aldehyde oxidase. A xanthinuric patient with normal formations of oxypurinol had normal aldehyde oxidase activity [24].

Urinary excretion of inosine has been reported to be increased in patients with xanthinuria [25].

The formation of xanthine is derived largely from guanine nucleotide degeneration under basal conditions and after intravenous fructose [25]. This would explain the prominence of xanthine of the oxypurines in hereditary xanthinuria. Of course, salvage of hypoxanthine via HPRT would also contribute to xanthine predominance.

TREATMENT

The absence of stone formation in many patients with xanthinuria is surprising. The relative insolubility of xanthine could make fluid intake irrelevant; it would nevertheless appear prudent to consume plenty of fluids.

Dietary manipulation and alkali are not helpful. In a patient with residual xanthine oxidase activity, allopurinol could be effective in converting some of the oxypurine excreted to the soluble hypoxanthine.

Allopurinol is effective in the treatment of gout and myeloproliferative diseases, by virtue of its effectiveness as an inhibitor of xanthine oxidase [25].

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73

Orotic aciduria

MAJOR PHENOTYPIC EXPRESSION

Megaloblastic anemia, failure to thrive, susceptibility to infection, crystalluria, orotic aciduria, and deficient activity of orotidylic pyrophosphorylase and orotidylic decarboxylase.

INTRODUCTION

Orotic aciduria was first reported by Huguley *et al.* in 1959 [1] in a single patient who illustrated clearly the features of the disease, particularly megaloblastic anemia. The rarity of the disease is indicated by the fact that it was 1965 before a second patient was described [2]. The importance of these two papers is reflected not only in the thoroughness of the clinical and metabolic documentation, but by the fact that they set out the definitive treatment with uridine [2, 3] which has been extraordinarily effective and serves as a model for the fact that the thorough understanding of the nature of a fundamental defect can lead to rational and effective treatment.

The disease is also special as an example of a defect in a single autosomal recessive gene causing defective activity of two sequential enzymes in the *de novo* pathway of pyrimidine nucleotide biosynthesis (Figure 73.1), orotidylic (OMP) pyrophosphorylase (OPRT) (EC 2.4.2.10) and orotidylic (OMP) decarboxylase (EC 4.1.1.23) [4, 5]. The gene on chromosome 3 has been sequenced, and a small number of mutations has been defined [6].

Patients have recently been reported in which orotic aciduria was not associated with megaloblastic anemia [7, 8]. They were considered to have a special type of orotic aciduria in which the ratio of orotic acid to orotidine approximated 1(range 0.46-1.31) as opposed to those with megaloblastic anemia in whom the ratio ranged from 5.9-26 [8]. More recently, we have reported [9] a patient with orotic aciduria without megaloblastic diarrhea in whom the ratio of orotic acid to orotidine was 15.85. Orotic aciduria without megaloblasticanemia was also reported by Grohmann *et al.* [10]. In their patient the ratio was 18.

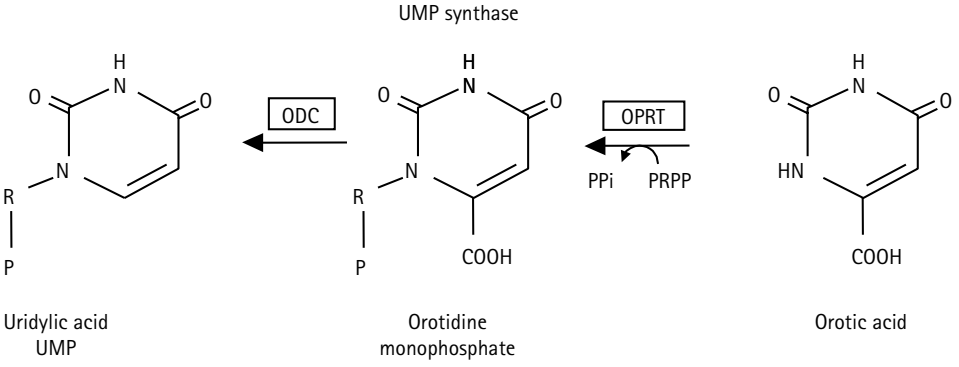

Figure 73.1 The pathway of pyrimidine nucleotide synthesis illustrating the enzymes OPRT and OMP decarboxylase components of UMP synthase that are defective in orotic aciduria.

CLINICAL FEATURES

The classic presentation (Table 73.1) [1, 2, 11] is with failure to thrive, but these infants are found to be pale and anemic on initial examination [1, 2, 5]. A few young infants presented with anemia in which growth had to date (two months) been normal [12]. A seven-year-old who developed symptoms of anemia at six years of age, but who had apparently been pale previously, had normal growth and intellectual development [13]. In the classic presentation, hair is sparse, fine, and very short [2]. Nails have grown poorly. One patient treated at 19 months with uridine had not had his nails trimmed for the previous six months [2].

The anemia is characteristically megaloblastic [1, 3, 5, 10–14]. Neutropenia is present in most patients. Hemoglobin levels have often been 7–8 g/dL, hematocrit approximating 25 percent, but some have had more severe anemia [14]. Some had required transfusions. Red cell morphology has been unusual, with a marked degree of anisocytosis and poikilocytosis [2], macrocytosis and many strikingly large and oval shapes with long diameters. Many macrocytes were hypochromic, while levels of iron are normal, or increased [5]. Occasional polychromatic cells have been seen, as well as strippled cells, Howell–Jolly bodies, Cabot rings, and nucleated erythrocytes. Multisegmented neutrophils and giant platelets have been observed [2]. Bone marrow aspirates reveal megaloblastic changes in a majority of the nucleated red cells. The myelo/erythroid ratio may be reversed to 1:2 or 1:4. Giant myelocytes and metamyelocytes are also seen.

Concentrations of B_{12} and folic acid are normal, and early patients were treated with B_{12} and folate without effect [1, 2]. Gastric aspirates did show achlorhydria, some of them responsive to histamine [2, 15] and some not [2].

The urine appears normal as passed, but on standing, especially in the cold, a large, white precipitate forms [1, 2, 12, 15]. Crystals of orotic acid may be visible under the microscope [2]. The disease was identified as an inborn error of metabolism by the recruitment by Huguley *et al.* [1] of a biochemist, J Bain, who isolated and purified the crystals and determined that they were orotic acid.

The crystalluria may lead to gross or microscopic hematuria. One patient [13] came to attention because of hematuria and was found to be anemic. Proteinuria is unusual, but has been found, along with urinary tract infection [13]. Oliguria may accompany infection, or decreased fluid intake or dehydration from some other source such as diarrhea [1]. Under such circumstances, there may be urinary tract obstruction because of orotic acid sludging in the ureters or urethra [1]. An intravenous pyelogram revealed a nephrogram effect with radiopaque material remaining in the kidney 1.5 hours after injection, presumably because of obstruction of the renal collecting tubules by crystalline orotic acid [12]. Urethral obstruction has been successfully relieved by catheterization [4]. Urea nitrogen and creatinine in blood are usually normal but will rise in the presence of obstruction.

Susceptibility to infection may be striking [1]. The initial patient had repeated respiratory infections and chronic diarrhea. He died of overwhelming varicella at 2.5 years [1]. An unusual feature was deficiency of specific immunoglobulins in one patient [12]. Others have had immunodeficiency, diarrhea, and stomatitis [16].

Congenital anomalies may or may not be a feature of this disease. Three of the first four patients reported had strabismus [2, 4, 11, 12] and one of these [11] had congenital heart disease. Another [4] had abnormal thoracic and abdominal musculature, herniation of lung into the supraclavicular region, umbilical and bilateral inguinal hernias, kyphoscoliosis, and a scaphoid skull. He also had hypertonia. Also, the published picture showed prominent genu recurvatum, and everted dorsiflexed feet in an exaggerated ballet first position.

Impaired physical and intellectual development has been observed [12, 17], but not invariably, since treatment has become available [3]. It has recently become apparent that there is another phenotype in which patients display developmental delay, in some relatively mild and no hematologic symptoms (Nyhan, unpublished observations) [16] (Figure 73.2). One had oculomotor apraxia. Orotic aciduria may be quantitatively less than in classic patients.

Table 73.1 Orotic aciduria clinical manifestations

Megaloblastic anemia	Crystalluria
Leukopenia	Hematuria
Susceptibility to infection	Obstructive uropathy
Failure to thrive	Urinary tract infection
Diarrhea	Nephropathy
Cognitive and motor developmental delay	

Figure 73.2 HA: A girl with orotic aciduria and UMP synthase deficiency. She had no abnormal hematologic findings. She had mild developmental delay, predominately of language, an abnormal EEG and episodes of cyclic vomiting and dehydration.

The orotic aciduria is usually massive. Excretion of as much as 1.34 and 1.5 g of orotic acid in 24 hours in a very young infant is not unusual [2]. The values approximate 1000 times the normal adult mean of 1.4 mg/24 hours. Orotidine excretion may also be elevated (15.8 mg/24 hours) as compared with the normal value of 2.5 mg/24 hours [8].

GENETICS AND PATHOGENESIS

The disease has been known to be autosomal recessive since early studies on four generations of heterozygous relatives of the first patient [18] in whom enzyme activity was demonstratively reduced.

The enzymatic deficiency of OPRT and OMP decarboxylase can be demonstrated in erythrocytes, leukocytes, and cultured fibroblasts [4, 5, 11], as well as in the liver [5]. Heterozygotes have intermediate level of activity, but they cannot always be reliably distinguished from control [4]. Initial distinction of types 1 and II orotic aciduria because of a patient in whom activity of OPRT appeared to be normal are clearly artificial in view of the fact that there is one gene and two enzymes. In fact, these early studies indicated that there were clear coordinate, straight-line relationships between the activities of the two enzymes in normal individuals and in patients [3]. Activities of enzymes earlier in the pathway, aspartate transcarbamylase and dihydroorotase, are elevated.

The defective enzyme, uridine-5-monophosphate (UMP) synthase contains in one polypeptide coded for by a single gene [6, 18] the activities of the two enzymes, which catalyze the last two steps of UMP synthesis [19].

The UMP synthase gene has been localized to chromosome 3q13 [20]. The gene contains six exons spanning approximately 15 kb. The protein contains 480 amino acids and has a molecular weight of 52,199. The two enzyme activities reside in distinct domains. The C-terminal 258 amino acids contain the decarboxylase and the N-terminal 214 the OPRT.

Two patients had a C378T missense mutation (P92S); in one a T961A mutation was also missense (I286N). In another family, two alleles contained R96G and G429A on one allele and V109G on the other [21]. Expression of human cDNAs containing these mutations in pyrimidine auxotrophic *Escherichia coli* demonstrated impaired enzyme activity. The patient with no megaloblastic anemia had a T928G mutation (I310V) [17].

TREATMENT

Orotic aciduria represents pyrimidine nucleotide starvation in man. It appears to be the first human nutritional auxotrophic disease to be recognized. The therapeutic effect of uridine is supportive of this hypothesis.

Excellent remission has regularly been obtained with doses of 50–300 mg/kg per day; some patients relapsed with less than 100 mg/kg, and only one required more than 200 mg/kg [16, 22, 23]. The dose most commonly employed is 150 mg/kg. Hematologic response is accompanied by weight gain and improvement in activity and well-being. Hair grows, and so do the nails. Orotic aciduria has now been treated with triacetyluridine the oral bioavailability of which is higher [24]. Peak concentration of uridine in plasma 1–2 hours after one single dose was 150.9 and that of repeated dosing 161.4. The level after the single dose was four-fold higher than that encountered with uridine. There were no adverse effects.

The conceptualization of effective replacement therapy began with the first publication [1]. Administration of uridylic and cytidylic acids led to reduction in orotic acid excretion. This was presumably a consequence of breakdown in the intestine to uridine and cytidine, as oral bioavailability of nucleotides is very low, and their administration usually results in diarrhea. Uridine therapy was initiated by Becroft and Phillips in the second patient [2]. Treatment begun at 16 months with 1.5 g/day led to a prompt rise in hemoglobin and a normal bone marrow. Activity and interest in his surroundings improved immediately, as did appetite. Hair and nails began to grow, as did he, crossing percentile lines for weight from below the 3rd percentile to between the 90th and 97th percentile. He remained mildly mentally impaired, but there was no progression. It was interesting that he experienced a prompt relapse on substitution of uracil for uridine, even though the content of pyrimidine base was twice that of uridine, which at that time was 75 mg/kg. Uridine therapy is dependent for bioavailability on efficient intestinal absorption and the activity of the salvage enzyme uridine kinase (EC2.7.1.48) which leads directly to the formation of the nucleotide UMP [25, 26].

6-Azauridine and 6-azauracil, used in cancer chemotherapy, must be converted to their nucleotides for them to have antitumor activity; consistent with the lack of effect of uracil in orotic aciduria, 6-azauridine is 20 times more effective as an antitumor agent than azauracil [27]. Growth of fibroblasts of a patient with UMP synthase deficiency in medium containing 6-azauridine displayed nearly normal levels of the two defective enzymes [28]. These observations, that an enzyme inhibitor may be therapeutic depending on interaction with the structure of the mutant protein or protection from degradation, could lead to therapy with azauridine, but uridine therapy has been so effective, it has not been tried.

Activities of aspartate transcarbamylase and dihydroorotase, which are elevated in the untreated patient, decrease with uridine therapy.

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74

Molybdenum cofactor deficiency

MAJOR PHENOTYPIC EXPRESSION

Developmental failure, intractable seizures, progressive loss of cerebral white matter, hypouricemia, elevated urinary S-sulfocysteine, loss of activity of xanthine oxidase, sulfate oxidase, and aldehyde oxidase; and mutations in MOCSIA, MOCSIB, MOCS2A, and the gene for gephyrin, GEPH.

INTRODUCTION

Molybdenum cofactor (MoCo) deficiency presents classically in the early neonatal period, with convulsions intractable to anticonvulsant therapy and coma. Without intubation and assisted ventilation, early neonatal death would be the outcome. Johnson and colleagues [1] reported in 1980 an extremely retarded girl in whom there was defective activity of both sulfite oxidase and xanthine oxidase. There were dislocated lenses. Urinary calculi were composed of xanthine, and excretion of hypoxanthine and xanthine were elevated. Urinary excretion of sulfite, thiosulfate, S-sulfocysteine, and taurine were increased. There was hypouricemia and decreased excretions of uric acid and sulfate. The primary defect was deficient synthesis of the MoCo.

The MoCo (Figure 74.1) consists of a small organic ring-structured compound known as molybdopterin joined by molybdenum which links the two sulfhydryls of the molecule (Figure 74.2).

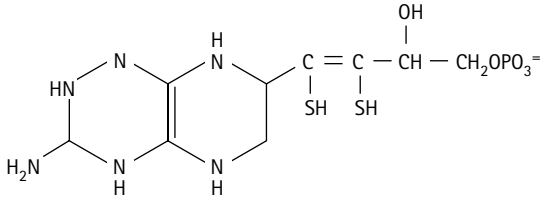

Figure 74.1 Molybdopterin, the molybdenum cofactor (MoCo).

The biosynthesis of this compound (Figure 74.2) involves three basic steps, the formation of precursor Z, catalyzed by two enzymes MOCS1A and MOCS1B, its conversion to molybdopterin, catalyzed by two enzymes MOCS2A and MOCS2B and activated by molybdopterin synthase (MOCS3), and the insertion of molybdenum, catalyzed by gephyrin (GEOH).

These enzyme proteins are coded for by four genes, MOCS1, MOCS2, MOCS3, and GEPH. Mutations anywhere in this pathway lead to the loss of activity of the three MoCo dependent enzymes, xanthine oxidase, sulfate oxidase and aldehyde oxidase (Figure 74.3). Mutations have been

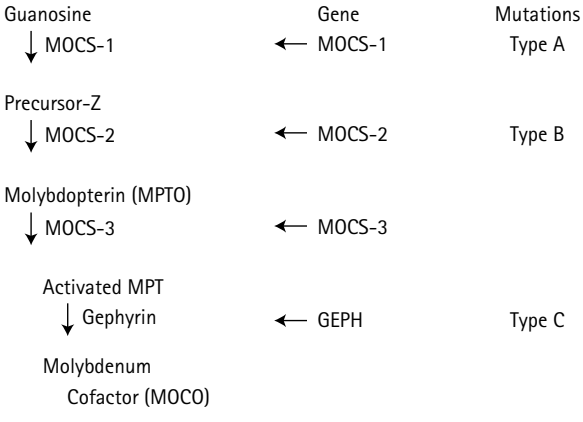

Figure 74.2 Genetically determined steps in the biosynthesis of the MoCo.

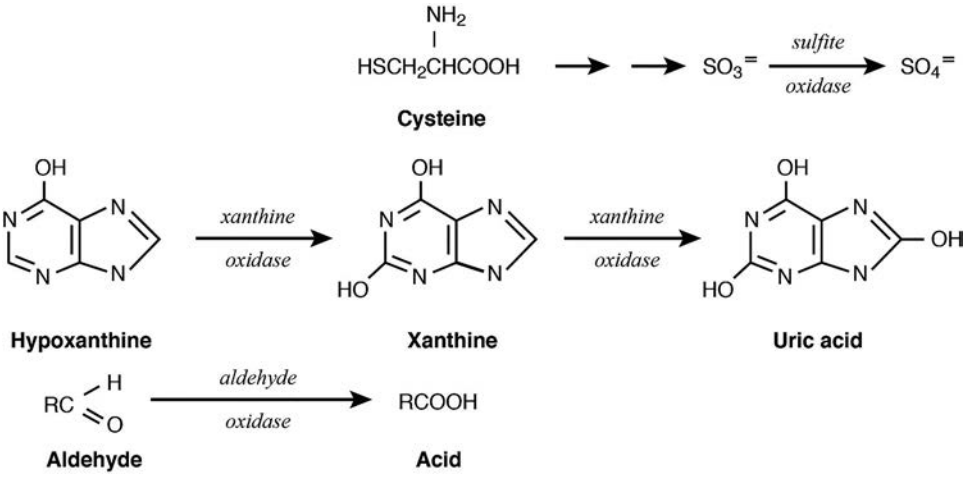

Figure 74.3 Role of the MoCo in the metabolism of cysteine, sulfate, xanthine and hypoxanthine.

identified in three of these diseases MOCS1, MOCS2, and GEPH [2] (Figure 74.4).

At least 32 different disease-causing mutations have been reported [2]. Most have been missense mutations, but there have been a few deletions, one insertion and two splice-site mutations leading to exon skipping. Among the most common mutations found in more than 5 alleles in Europe were p.R73W in MOCS1A, p.R319Q in MOCS1A, c.1523-1524del AG in MOCS1B and c.726delAA in MOCS2B.

CLINICAL ABNORMALITIES

Our patient (Figure 74.5) appeared well for two days after an uneventful pregnancy and delivery, when he suddenly appeared dusky mottled and floppy, and shortly after, he became apneic and required assisted ventilation. EEG revealed status epilepticus and there was little response to anticonvulsant therapy. By ten days of age, there was evidence of cerebral atrophy. By the end of the first year he not only had microcephaly, but overriding sutures. There was no evidence of neurologic development. Axial hypotonia may coexist with hypertonia and spastic tetraparesis. This is the classic presentation. Cerebral atrophy is evident on neuroimaging. Decreased density of the white matter is also seen. Many patients have died in infancy.

Dislocation of the lenses may be a late finding, and some infants have died without demonstrating this finding. A patient reported [3] developed dislocation of the lenses at eight years of age. Other ocular findings seen earlier in this patient included spherophakia, small spherically lenses prone to subluxation, typically seen in the Weill-Marchesani syndrome, but the authors attributed subluxations to abnormal relaxation of the zonular fibers that ultimately leads to dislocation.

Some patients have had less dramatic presentations, but a severe degree of developmental retardation has been the rule. Convulsions, poor feeding at least in the early weeks and months of life, and dislocated lenses are features shared by MoCo deficiency and isolated sulfite oxidase deficiency, but the former is turning out to be much more common, or less rare, than the latter [4]. The authors called attention to the usefulness of a stick test for sulfite in the urine in the detection of both diseases. On the other hand, a patient with the classic presentation had negative stick sulfite tests [5]. The authors held that hypouricemia is a better alerting signal for the presence of MoCo deficiency. Another marker for the disease is the absence of urothione,

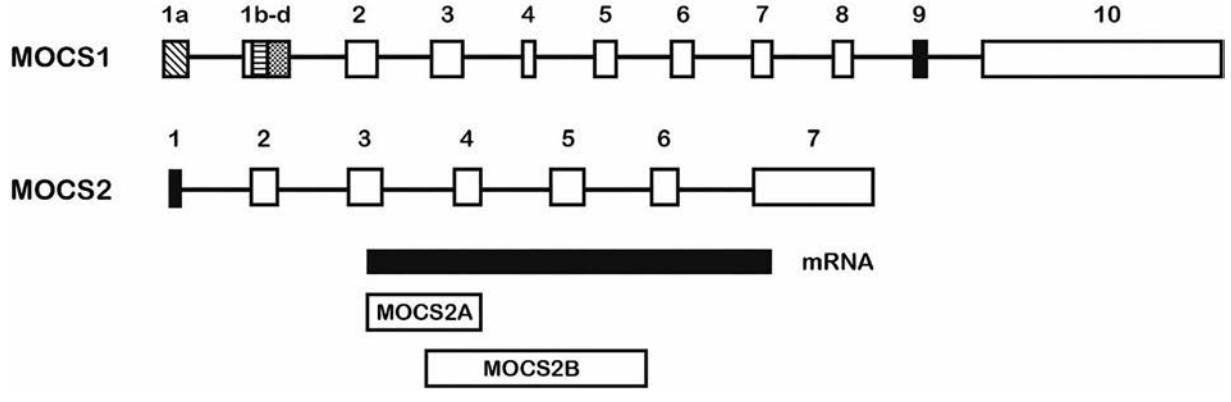

Figure 74.4 The MOCS1 gene; above and the MOCS2 gene below; exon numbers are given above. Exon 9 in MOCS1 is dark to indicate its potential deletion.

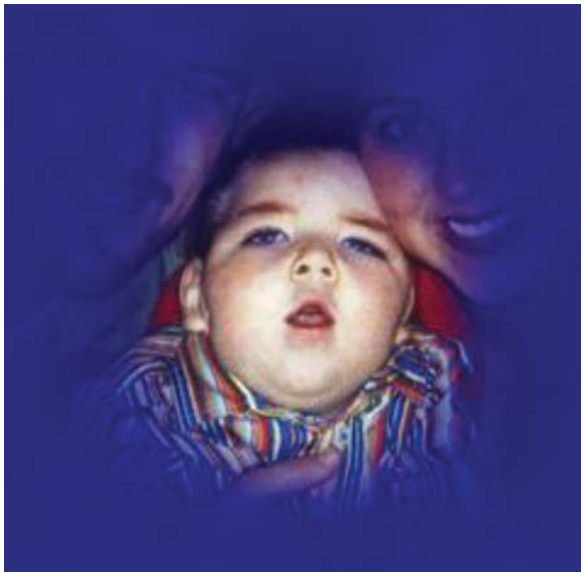

Figure 74.5 RM, an infant with MoCo deficiency; he had two mutations in the *MOCS1A* gene, p.R67W (c.CGG>TGG in exon 1), and p.G126D(c.GGT>GAT in exon 2). The first was a novel mutation in a highly conserved position; the latter had been identified in several European and American patients. He had become apneic and required ventilation on the second day of life. EEG revealed status epilepticus and later a burst suppression pattern. He was ultimately weaned from the ventilator, but showed little sign of neurologic development.

a normal degradation product of the MoCo, and a sulfur containing pterin [6]. This observation was confirmed in another patient with the disease [7]. Additional features of the abnormal metabolic milieu of this disease include large amounts of sulfite, taurine, S-sulfocysteine, and thiosulfate, indicating deficiency of sulfite oxidase, as well as increased excretion of hypoxanthine and xanthine resulting, like the hypouricemia from xanthine oxidase deficiency [8]. These authors documented deficient activity of both of these oxidase enzymes [8]. The importance of hypouricemia in suggesting the diagnosis has also been emphasized [9].

Deficiency of sulfite oxidase may be demonstrated in cultured fibroblasts [10]. Xanthine oxidase is not expressed in fibroblasts. Deficiency of its activity was demonstrated in biopsied liver [8].

In coculture studies of fibroblasts, two complementation groups were discovered and designated A and B [10]. Conditioned media from cells of type B demonstrated correction of defective activity in type A cells and revealed the presence of a stable diffusible factor. This factor was related to molybdopterin in mutants of Neurospora and *E. coli*.

GENETICS AND PATHOGENESIS

All of the MoCo disorders are inherited as autosomal recessive diseases [2]. Consanguinity has been observed. More than 100 patients have been diagnosed, and some 50 families representing all ethnic groups have been studied at the molecular level. It is thought that incidence is likely to be higher; these patients often die very young, many probably without diagnosis. Prenatal diagnosis has been accomplished [11–13]. This can be done without culture in chorionic villus material.

The genetics of MoCo deficiency were first aided by the identification of the two complementation groups by coculture of fibroblasts from different patients [10]. Two thirds of those studied were placed in group A and deficient in the early steps of the synthesis of precursor Z (see Figure 74.2). One third were placed in group B representing the conversion of precursor Z to molybdopterin.

Two genes coding for the synthesis of MoCo were first cloned from Arabidopsis thaliana [14]. Search for homologous sequences led to the identification of *MOCS1* [15]. The gene is bicistronic and codes for the first two proteins in the formation of precursor Z, MOCS 1A and 1B. Mutations were found in both cistrons [15, 16]. The gene is located on chromosome 6.

The gene has a complicated splicing pattern generating three different isoforms with two exclusive start codons [16]. Alternate splicing in exon 9 also yields three isoforms [17]. The cDNA has two open reading frames separated by A stop codon within exon 9. Only the MOCS1A protein translated from the mRNA is functional. The second and third isoforms code for MOCS1B [18].

The *MOCS2* gene was found by homology search and localized to chromosome 19. It also codes for two molybdopterin synthase proteins; they sequentially convert precursor Z to molybdopterin. Mutations have been found in both MOCS2A and 2B [20, 21].

The synthesis of molybdopterin requires an activation by a sulfotransferase which is coded for by an intronless *MOCS3*. Mutations have to date not been found in this gene [22].

The gene for gephyrin is on chromosome 14 [2]. Gephyrin had been found previously as a neurotransmitter receptor clustering protein [23] for the glycine receptor in spinal cord. Patients with this deficiency are in complementation group B. Fibroblasts from a patient with this defect were shown to activate sulfite oxidase by incubation with large amounts of molybdenum [2] One family whose children died had a deletion of exon 2 and 3 of the gephyrin gene leading to frameshift and a premature termination codon in exon 4 [2].

Among MOCS1 mutations, 13 known compound heterozygous mutations accounted for 50 percent of all MoCo deficiencies [2, 24]. The majority were in the MOCS1A domain. Two of three deletions abolished the highly conserved C-terminus; this would not permit expression. Among the MOCS1B mutations an insertion c.1313insG and the deletion c.1523delAG lead to frameshifts; nonsense mediated mRNA decay [25] has been suggested [2].

Most MOCS1 mutations found in more than one family showed concentration in part of the world [2] suggesting

founder effect. Each parent was regularly shown to have one of the abnormal alleles. No de novo mutation has yet been described. MOCS2 mutations [19] were also localized to a single open reading frame. Two were in the MOCS2A domain and three in the MOCS2B domain. There were three stop codons, Q6X, Q30X and Q36X, as well as the start codon mutation c.3G>A in which disruption of translation initiation abolished expression [19]. Deletions were common in MOCS2. A frameshift mutation c.726 del AA was the most common mutation in MOCS2B, accounting for 11 of 28 alleles.

The clinical manifestations of MoCo deficiency relating to the nervous system are generally considered to be functions of the deficiency of sulfite oxidase. They are similar to those of isolated sulfite oxidase deficiency. Deficiency of xanthine oxidase leads to elevations in levels of xanthine, as well as hypouricemia. Xanthine is highly insoluble, and this leads to urinary tract calculi, hematuria, renal colic, and infection.

TREATMENT

Convulsions are treated with the usual anticonvulsant drugs, but response is seldom very good. The usual supportive therapy is employed.

Therapy with molybdate has been employed without success. However, none of the patients treated were defined at the molecular level; it is likely that they represented MOCS1 or 2 mutations. A patient with gephyrin deficiency might be different, but synaptic abnormalities resulting from the receptor clustering effect of this gene makes it less likely.

Precursor Z was isolated from bacteria and found to be more stable than molybdopterin; it was overproduced in *E. coli*, purified and used successfully to treat knockout mice [26].

Six patients with MoCo deficiency have been treated with this intermediate, now known as cyclic pyranopterin monophosphate [27]. All were type A and had mutations in MOCS1 which causes a loss of cyclic pyranopterin monophosphate (cPMP), the first intermediate in the pathway of molybdopterin synthesis. Patients were diagnosed *in utero* or between two and 20 days of age. Treatment was begun on days 0–20 in daily doses of 80–240 μg/kg intravenously. Within days all of the urinary markers of the disease, sulfite, sulfocysteine, thiosulfate, xanthine, and hypoxanthine became normal or nearly so. Infants became more alert, and seizures stopped. Follow up of two treated children who had the same mutation in MOCS1, in whom treatment was started on days five and seven of life was reported [28] when they were 15 and 18 months of age. One had very "satisfying" development. The other had dystonic cerebral palsy and cystic encephalopathy.

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PART 9

MUCOPOLYSACCHARIDOSES

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Introduction to mucopolysaccharidoses

The mucopolysaccharidoses are genetically determined disorders in which acid mucopolysaccharides, known chemically as glycosaminoglycans, are stored in the tissues [1, 2] and excreted in large quantities in the urine [3]. Storage in tissues leads to effects on a wide variety of systems and to remarkable changes in morphogenesis. Among these striking effects are the alterations in the appearance of the patient that are classically represented in Hurler syndrome (Chapter 76). The elucidation of these disorders has provided clear evidence that even bizarre dysmorphic changes can be caused by single gene defects that interfere with body chemistry. They provide important models of the interaction of structure and function in humans. Mental retardation and early demise, prior to ten years of age in Hurler syndrome, are the most devastating consequences of mucopolysaccharide accumulation in the central nervous and cardiovascular systems.

However, there is considerable variety of expression among patients with various individual mucopolysaccharidoses. Patients with some syndromes are intellectually normal, and some survive well into adult life. Attenuated forms of mucopolysaccharidosis may be undiagnosed for many years. The importance of delayed diagnosis is further heightened by the preventive nature of most enzyme replacement therapy.

Research in this field has proceeded rapidly, so that it is now possible to delineate the molecular defect at the level of the enzyme in each of the mucopolysaccharidoses, and the genes of most of them have been cloned and mutations in them identified.

Advances in the understanding of the mucopolysaccharidoses followed the growth of fibroblasts from these patients in cell culture and the recognition that there was phenotypic expression of the disease in the fibroblast. The elucidation of the molecular nature of the mucopolysaccharidoses represents a fascinating chapter in cell biology. Characterization of the mucopolysaccharidoses as disorders in the degradation of intracellular acid mucopolysaccharide began with the studies of Fratantoni, Hall, and Neufeld [4] using ^{35}S-labeled sulfate. $^{35}SO_4$ is taken up by the cells of patients, just as it is by normal cells. However, in patients as opposed to controls, there is no turnover; these cells simply accumulate the label and keep it.

In what is now a landmark series of experiments, Fratantoni, Hall, and Neufeld [5] mixed normal fibroblasts in culture with those of patients with Hurler or Hunter syndrome and found that the kinetics of $^{35}SO_4$ incorporation became normal. Furthermore, it was possible to restore normal kinetics in Hurler cells by mixing them with Hunter cells and vice versa. It was also found that the medium in which normal cells or Hunter cells had grown could correct the defect in Hurler cells. Corrective factors were soon identified for other mucopolysaccharidoses [6]. In fact, demonstration of two different corrective factors first permitted the distinction of Sanfilippo types A and B. On the other hand, fibroblasts from patients with Scheie syndrome could not be corrected by the factor from Hurler cells [7], indicating that the genes for these two conditions were allelic and represented different defects in the same enzyme protein. These studies in cell biology led directly to the identification of the enzymatic defects [8, 9] (Table 75.1). They also laid the groundwork for current enzyme replacement therapy with recombinant enzymes.

Hurler disease was originally classified by McKusick as mucopolysaccharidosis type I [1]. With the recognition of the enzyme defect in α-L-iduronidase and the fact that defective activity of the same enzyme was also the cause of the Scheie syndrome [8], the subclassifications IH for Hurler and IS for Scheie were employed. The classification of the mucopolysaccharidoses and a summary of their clinical biochemical characteristics are shown in Table 75.1. The seven types of mucopolysaccharidosis represent the deficiencies of eleven specific enzymes. Prenatal diagnosis was initially carried out in Hurler and Hunter diseases by measuring labeled sulphate incorporation in cultured amniocytes [10].

The defect in the Hurler cell is in α-L-iduronidase [8, 9, 11] (Figure 76.1), and Hurler corrective factor has been shown to have iduronidase activity [8]. The Hurler corrective factor is a form of iduronidase that can be taken up by fibroblasts [12] because it contains the mannose-6-phosphate recognition marker, whereas the lower molecular weight enzyme purified from human kidney cannot. The mucopolysaccharidoses I through VII represent defective activity in the enzymes required for the stepwise degradation of heparan sulfate, dermatan sulfate, keratan

Table 75.1 Clinical and laboratory characteristics of the mucopolysaccharidoses

Syndrome	MPS Designation*	Inheritance	Mental retardation	Corneal clouding	Hepatosplenomegaly	Skeletal defect	Other clinical manifestations	Glycosaminoglycans stored excreted	Defective enzyme
Hurler	I_S	Autosomal recessive	+	+	+	+	Coarse facial features, cardiac disease, motor weakness, hernia	Dermatan sulphate heparan sulphate	α-L-iduronidase
Scheie*	I_{HS}	Autosomal recessive	–	+	–	+	Coarse features, stiff joints	Dermatan sulphate heparan sulphate	α-L-iduronidase
Hurler/Scheie	I_S	Autosomal recessive	±	+	+	+	Phenotype intermediate	Dermatan sulphate heparan sulphate	α-L-iduronidase
Hunter	II	X-linked recessive	+	–	+	+	Coarse features, weakness, aggressive behavior	Dermatan sulphate heparan sulphate	Iduronate sulfatase
Sanfilippo Type A	III_A	Autosomal recessive	+	–	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulphate	Heparan N-sulfatase (sulfamidase)
Sanfilippo Type B	III_B	Autosomal recessive	+	–		+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulphate	α-N-Acetylglucos-aminidase
Sanfilippo Type C	III_C	Autosomal recessive	+	–	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulfate	AcetylCoA: a-D-glucosaminide-*N*-acetyl transferase
Sanfilippo Type D	III_D	Autosomal recessive	+	–	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulfate	*N*-Acetyl-α-D-Type D glucosaminide-6-sulfatase
Morquio A	IV_A	Autosomal recessive	±	+	–	+	Distinctive bone deformities, hypoplastic odontoid, thin enamel	Keratan sulfate	Galactose-6-sulfatase
Morquio B	IV_B	Autosomal recessive	±	+	+	+	Mild bone changes, hypoplastic odontoid	Keratan sulfate	β-Galactosidase
Maroteaux- Lamy	VI	Autosomal recessive	–	+	+	+	Severe bony deformities, valvular cardiac disease	Dermatan sulfate	Acetylgalactosamine 4-sulfatase (arylsulfatase β)
Sly	VII	Autosomal recessive	+	+	+	+	Coarse features	Dermatan sulfate heparan sulfate, chondroitin-4-6-sulfates	β-Glucuronidase
	IX	Autosomal recessive	+	–	–	+	Periarticular soft tissue masses; short stature	Hyaluronan	Hyaluronidase

*Types V and VIII have become obsolete.

sulfate, or chondroitin sulfate. The gene and the cDNAs for the enzymes defective in the mucopolysaccharidoses with the exception of the MPS IIIC enzyme have been mapped to their respective chromosomes [13] and cloned, and many mutations have been identified.

The diseases are autosomal recessive except for Hunter disease which coded for on the X chromosome [14]. In the absence of effective enzyme activity, the glycosaminoglycans are stored in the lysosomes.

A suspected diagnosis of mucopolysaccharidoses is often pursued chemically by the documentation of increased amounts of mucopolysaccharide in the urine. However spot tests for mucopolysaccharide are unreliable and give false positive and negative results [15]. Semiqualitative and quantitative procedures may also be misleading [16]. If a diagnosis of a mucopolysaccharidoses is suspected, assay of the lysosomal enzymes should be performed. This is readily carried out in freshly isolated leukocytes. It can also be done on cultured fibroblasts.

A common feature among the mucopolysaccharidoses is the roentgenographic appearance [17, 18] known as dysostosis multiplex. This picture is best exemplified in Hurler disease. This is such a constant feature of the disease that roentgenographic search for the presence of dysostosis multiplex is an effective way to screen for the mucopolysaccharidoses. It is reliable in all but the Sanfilippo patients, and it is most dramatic in the Hurler patients. This picture is also seen in generalized GM_1 gangliosidosis and in the mucolipidoses (Chapter 83). It is described in detail in Chapter 76.

Therapy has been successful via bone marrow transplantation in some of these diseases. Umbilical cord transplantation has had limited success and neurologic outcome has not been favorable for either. Earlier diagnosis has been emphasized [19, 20] as critical for success of ERT. Enzyme replacement therapy has been of mixed efficacy, but certainly there are effects on some features of the diseases. A variety of supportive measures, such as surgical fusion to stabilize a hypoplastic odontoid can be of great benefit.

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Hurler disease/mucopolysaccharidosis type IH (MPSIH)/α-L-iduronidase deficiency

MAJOR PHENOTYPIC EXPRESSION

Coarse features, mental retardation, corneal clouding, hepatosplenomegaly, short stature, dysostosis multiplex and cardiac complications; widespread lysosomal storage of mucopolysaccharide, and excretion of dermatan sulfate and heparin sulfate; and deficiency of α-L-iduronidase.

INTRODUCTION

Hurler syndrome is the classic or prototypic mucopolysaccharidosis (MPS). Hurler's original description was published in 1919 [1]. McKusick classified it as MPS I [2], and more recently as IH to distinguish it from the Scheie phenotype IS, or the intermediate Hurler-Scheie (IHS) picture. Modern molecular biology makes these distinctions less relevant, but we have continued to separate MPS I into two chapters because of the importance of these phenotypes and because these distinctions may have relevance to therapy.

The defect in the Hurler cell is in α-L-iduronidase [3–5] (Figure 76.1). The gene for α-L-iduronidase has been mapped to chromosome 4p16.3 [6] and has been cloned and sequenced [7]. A number of mutations have been identified, including at least two common alleles W402X and Q70X, accounting for over half the alleles in European patients [8–10]. Heterogeneity is also evident in different mutations in other ethnic groups [11, 12].

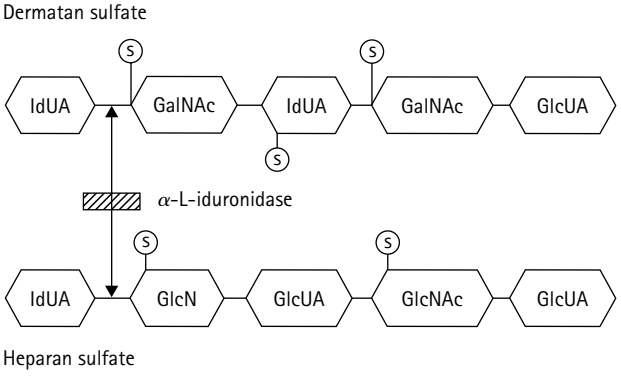

Figure 76.1 α-L-iduronidase, the site of the defect in Hurler and Scheie diseases. Dermatan sulfate and heparan sulfate accumulate when α-L-iduronidase activity is defective.

CLINICAL ABNORMALITIES

Patients with Hurler syndrome appear normal at birth. They develop normally for some months, after which, they begin to develop progressive disease. Patients may present first for repair of inguinal hernias or for chronic rhinitis [13]. The diagnosis is seldom suspected at that time. However, as the first year of life proceeds, the characteristic appearance develops. Nasal discharge tends to be persistent, as are recurrent respiratory infections and otitis. Breathing is noisy, as is snoring.

In the established syndrome, the facial features are coarse (Figures 76.2–76.6). The head is large, bulging and scaphocephalic, and there may be hyperostosis of the sagittal sutures. Frontal bossing, prominent brow, wide-set prominent eyes with puffy-appearing lids and a depressed

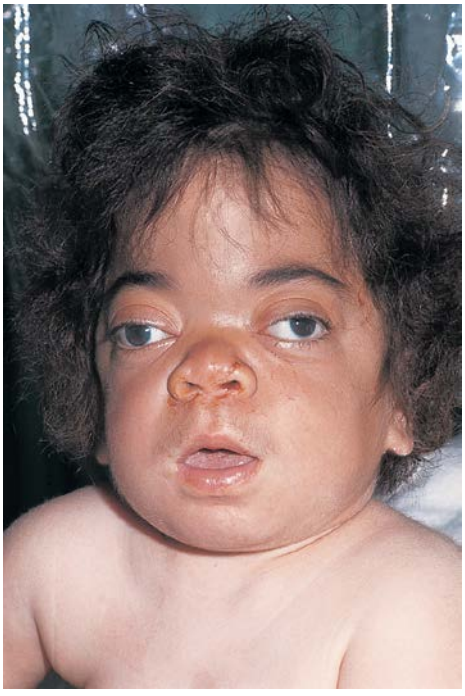

Figure 76.2 DD. A six-year-old girl with Hurler disease. She was short (90 cm) at the age of seven and had a relatively large head (55 cm). The facial features were coarse, the eyes were prominent and the nasal bridge depressed. There was frontal bossing. The abdomen was protuberant because of hepatosplenomegaly, and there was an umbilical hernia.

Figure 76.3 MOMMR. This toddler with Hurler disease illustrates the evolution of the disease. The abdomen was protuberant and facial features coarse, but much less than in Figure 76.2.

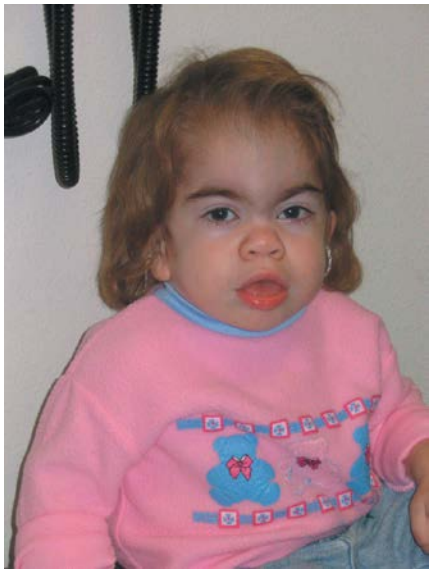

Figure 76.4 SC. This two-year and ten-month-old with α-iduronidase deficiency had clear cut dysostosis multiplex, and her hand was the typical claw hand, but her facial features were subtle. The alae nasi and septum had begun to widen, and she was quite hirsute; corneas had begun to cloud.

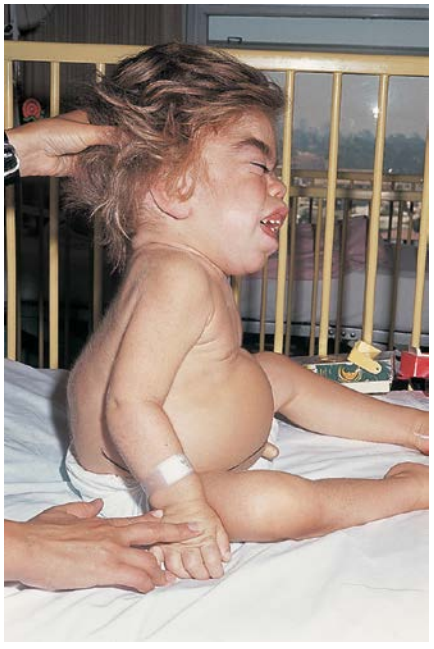

Figure 76.5 BR. This five-year-old girl with advanced Hurler disease had massive hepatosplenomegaly and a gibbus.

nasal bridge are characteristic. The face is flat, and the nose and nostrils wide and anteverted (Figures 76.2, 76.5, 76.6). The lips are large and thickened; the tongue is large and often protrudes through the open mouth (Figure 77.11). There is hypertrophy of the gums and the bony alveolar ridges; the teeth are small and widely spaced. Patients are generally hirsute. The hair is thick and coarse, the eyebrows

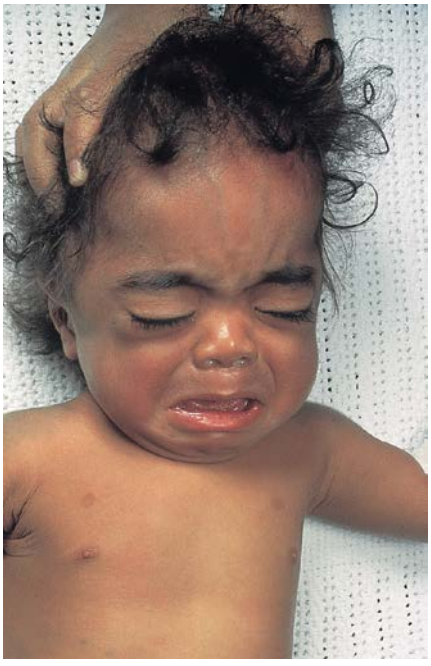

Figure 76.6 GAN. An infant with pronounced stigmata of Hurler disease. The facial features were quite coarse as early as five months. Activity of α-L-iduronidase was undetectable.

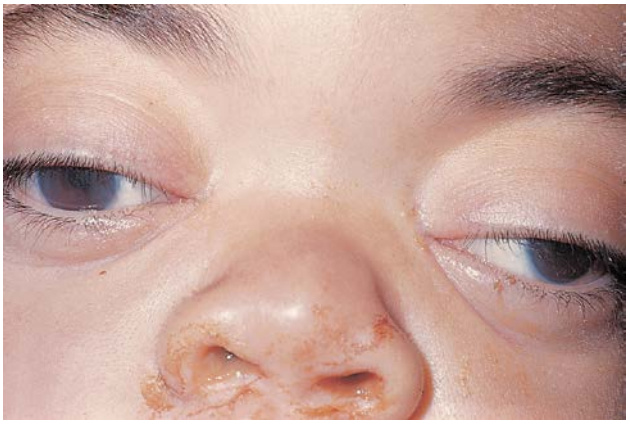

Figure 76.7 DD. The corneas were steamy.

bushy and the hairline low, and there is a large amount of forehead hair. Lanugo hair is plentiful. The skin is thick.

Clouding of the cornea is a hallmark of the syndrome (Figure 76.7). The cloudy cornea has a ground-glass appearance. It may lead to blindness. Nystagmus and strabismus are occasionally seen. Some patients develop glaucoma [14]. Sensorineural or mixed conductive and neural deafness develops regularly.

Developmental delay may be evident within the first 12 months, but intellectual deterioration is progressive to a level of severe impairment. The peak of intellectual function may be about two to four years of age or earlier, after which there is a steady regression. Behavior is usually quite pleasant, and these are often lovable children despite their unusual appearance.

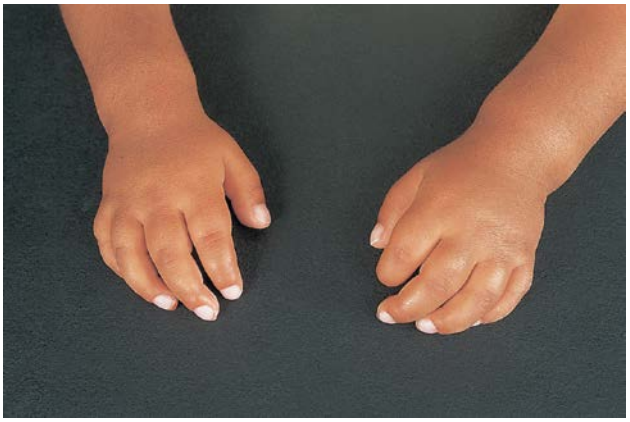

Figure 76.8 The typical claw hands of a patient with Hurler disease. Limitation of motion is evident in the position of the digits.

Shortness of stature is characteristic. Linear growth appears to stop at two to three years of age. Maximum height in one large series was 97 cm [13]; few exceed 100 cm. The neck is short, and the large head appears to rest directly on the thorax. The lower rib cage flares. The back is kyphotic, and there is a gibbus in the lower thoracic or upper lumbar area (see Figure 76.5). The joints become stiff, and mobility may be severely limited, especially at the elbows. The hands become broad, and the fingers stubby. This, and the limitation of extension and the position in flexion, produces the characteristic claw hand (Figure 76.8). The abdomen is protuberant. The liver and spleen become very large and very hard. Umbilical hernias are the rule, and inguinal hernias and hydroceles are common. Recurrence of a hernia is frequent following surgical repair. In a study on Hurler patients following hematopoietic stem cell transplantation there was a high incidence of pronated posture, foot and ankle disability, and a requirement for customized footwear [15].

Cardiac complications are prominent late features of the disease and often represent the cause of death. Some patients have been reported in whom acute cardiomyopathy and endocardial fibroelastosis were evident in the first year of life [16, 17]. These are infantile cardiac manifestations. Later cardiac disease is valvular; murmurs, aortic regurgitation, and mitral or tricuspid atresia result from storage of mucopolysaccharide in the valves. These features lead to congestive cardiac failure. Thickening of the valves of the coronary arteries leads to angina pectoris and myocardial infarction. Coronary angiography may underestimate the degree of involvement [18]. Endothelial dysfunction and reduced activity of nitric oxide synthase has been documented by peripheral artery tonometry [19]. Immunohistochemical staining study of coronary arteries stenotic, as a consequence of myointimal proliferation, displayed hyperactive transforming growth factor-beta (TGF-β) [20]. TGF-β has been related to pathogenesis of cardiovascular disease including hypertrophic cardiomyopathy. These observations could lead to a new

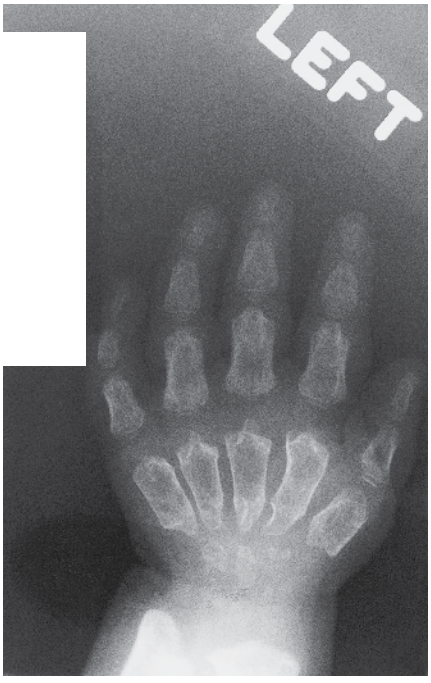

Figure 76.9 DD. Dysostosis multiplex is seen classically in the bones of the hand. The radial and ulnar articular surfaces are angulated toward each other. Marked irregularity and retarded ossification of the carpal bones are seen as well as coarsening of the trabeculae of the phalanges and metacarpals. The metacarpals are broadened at their distal ends and tapered at the proximal ends with a hook-like deformity. The phalanges, especially the distal ones, are short and the proximal and middle phalanges are characteristically thick and bullet shaped.

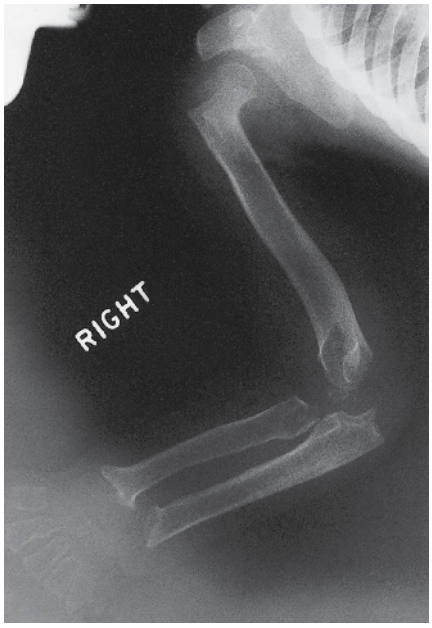

Figure 76.10 DD. The long bones of the upper extremity illustrate the lack of normal modeling and tabulation of the diaphyses, making these bones short and stubby. There was a varus deformity of the humerus. The ulnar semilunar notch was shallow and the radioulnar inclination abnormal.

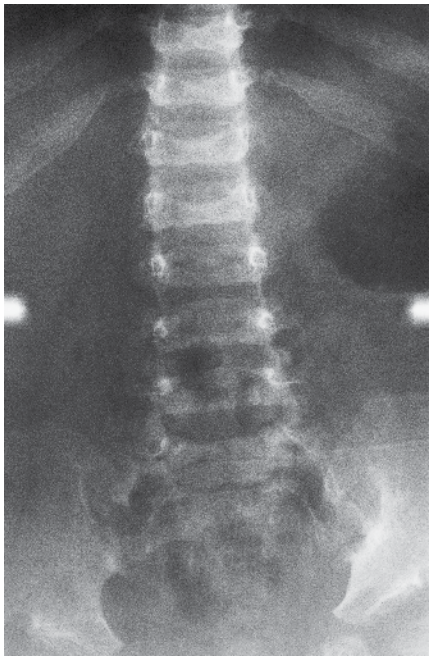

Figure 76.11 DD. The roentgenographic appearance of the ribs was classic. The spatulate shape is caused by a generalized widening of the ribs, which spares the relatively narrow proximal portions.

therapeutic approach with agents which lead to suppression of TGF-β signal.

Patients may also die of pneumonia. They tolerate anaesthesia very poorly [13].

The roentgenographic appearance of dysostosis multiplex in these patients is classic (Figures 76.9–76.14) [21, 22] The shafts of all the bones widen. The cortical walls become thickened externally during the first year of life, but later they become thin as the medullary cavity dilates. Lack of normal modeling and tubulation characterizes all the bones (Figures 76.9 and 76.10). Epiphyseal centres are poorly developed. The bones of the upper extremities become short and stubby (Figure 76.10) and taper toward the ends, often with enlargement of the mid-portions. The ends of the radius and ulna angulate toward each other (Figure 76.10). The roentgenographic appearance of the claw hand (Figure 76.9) of the patient with Hurler syndrome is pathognomonic of dysostosis multiplex. The metacarpals are broad at their distal ends and taper at their proximal ends. The phalanges are thickened and bullet shaped. The lower extremities show moderate enlargement of the shaft. There may be coxa valga, small femoral heads, and a poorly developed pelvis. The lower ribs are broad and spatulate (Figure 76.11). The clavicle is absolutely characteristic, while the lateral portion may be hypoplastic or even absent. The vertebrae are hypoplastic, scalloped posteriorly, and beaked anteriorly, especially at the thoracolumbar junction (Figure 76.12). In this area, there is anterior vertebral wedging, and this leads to the thoracolumbar gibbus, typically with a hooked-shaped vertebra at the gibbus. Hypoplasia of the odontoid may

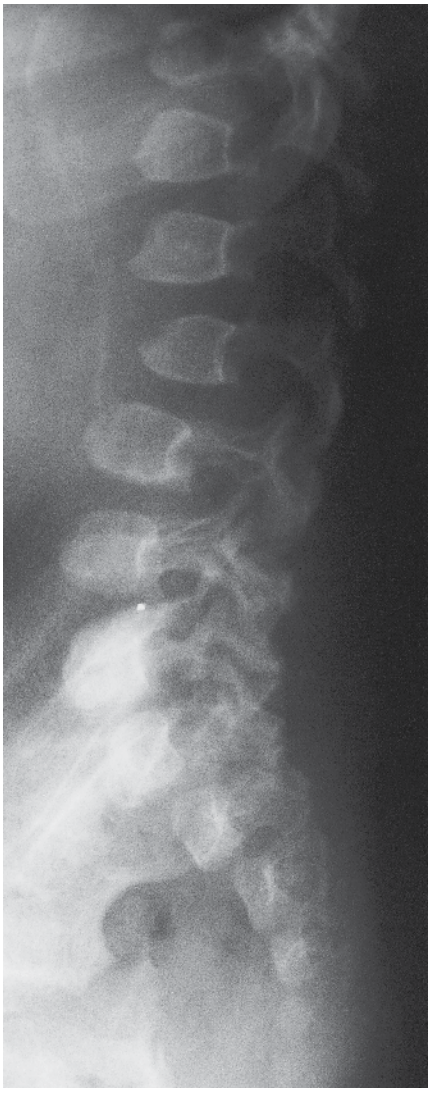

Figure 76.12 Roentgenographic appearance of the spine of DD. The antero-posterior distance was diminished in the vertebral bodies, and there was marked posterior scalloping. The pedicles of the lumbar spine were elongated. There was a marked thoracolumbar gibbus and inferior beaking of T12, L1 and L2.

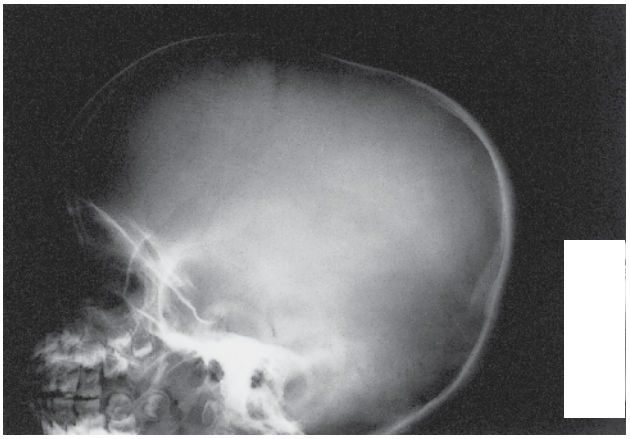

Figure 76.13 SMQ. Roentgenogram of the skull illustrates the early appearance of the J-shaped sella turcica.

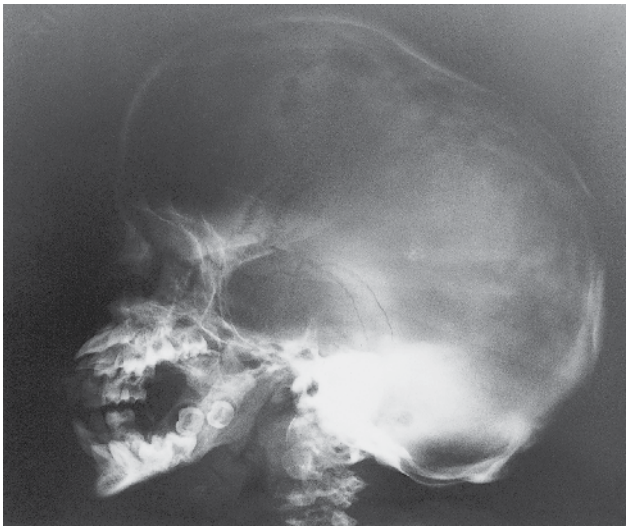

Figure 76.14 Roentgenographic appearance of the skull of DD. The very large cranium of both occipital and frontal areas was prominent. There was calcification of the choroid plexus of the left lateral ventricle. There was enormous enlargement of the sella and erosion of the clinoid processes. The mandibular rami were short, and there was increased angulation at the junction of the body and the varus, as well as flattening of the condyles.

be present, and this can lead to atlantoaxial subluxation, as in Morquio disease (Chapter 80). The skull is large, the orbits shallow and the sella turcica shoe-shaped or J-shaped (Figures 76.13 and 76.14).

Complications include cord compression, hydrocephalus, and pigmentary degeneration of the retina. Death usually occurs by ten years of age. At autopsy, the weight of the brain is increased, indicating that the increase in head size is a consequence of the storage of material. Thickening of the meninges is also seen. It is for this reason that some patients develop hydrocephalus. Pachymeningitis in the cervical area may also lead to myelitis or spinal nerve root compression. Electron-microscopic examination of the brain reveals the presence of zebra bodies resembling those of Tay-Sachs disease (Chapter 88). These findings have been interpreted as indicating the accumulation of ganglioside in brain [23], and this has been documented chemically [24]. Large vacuolated cells are found in many tissues.

Characteristic granules (Reilly bodies) are found in the polymorphonuclear and other leukocytes (Figure 76.15). The mucopolysaccharide found in tissues such as the liver and spleen is dermatan sulfate [24, 25]. Large quantities of dermatan sulfate and heparan sulfate are excreted in the urine. In Hurler syndrome, these two compounds are excreted in an approximate ratio of 2:1. Mucopolysaccharide also accumulates in the brain. Metachromasia may be demonstrated in cultured fibroblasts by a pink stain with toluidine blue [26]. Quantitative analyses have revealed increased amounts of dermatan sulfate in fibroblasts of patients with Hurler syndrome [27].

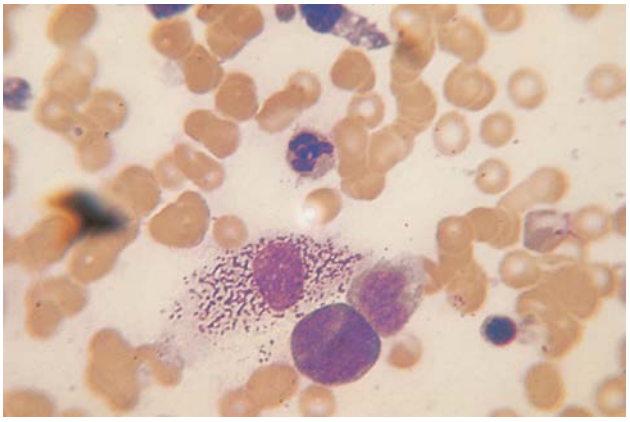

Figure 76.15 Bone marrow illustrating the Reilly bodies of Hurler syndrome. The histiocytes in the center of the field were full of these inclusions. (This illustration was kindly provided by Dr Faith Kung of the University of California San Diego.)

GENETICS AND PATHOGENESIS

Hurler syndrome is determined by an autosomal recessive gene. Parental consanguinity has commonly been reported. The incidence of the disease in a British Columbia survey was estimated at one in 144,000 births [28].

The molecular defect in Hurler disease is in the activity of α-L-iduronidase (see Figure 76.1) [3, 4, 5]. This enzyme catalyzes the hydrolysis terminal iduronic acid residues of dermatan sulfate and heparin sulfate. The enzyme has been purified from the human liver, kidney, and lung [29–31]. The cDNA codes a protein of 653 amino acids [32]. The protein exists as a monomer of 70 kDa minus the signal sequence [33]. There are six potential sites for N-glycosylation. It acquires mannose-6-phosphate, which permits targeting to lysosomes [33]. The deficient activity of enzyme is readily demonstrated in cultured fibroblasts and in leukocytes [34–37]. Residual activity of the enzyme has not been useful in distinguishing variants with phenotypes of greater or lesser severity, including the Scheie syndrome. Also, immunochemical studies have not been helpful with these distinctions.

Carrier detection can be performed by assay of the enzyme in cultured fibroblasts or in freshly isolated leukocytes, in either of which, activity of iduronidase is about half that of normal cells [38, 39]. However, the ranges of activity in both normal and carrier populations are so great that it may be difficult to ascertain for certain that any individual is a non-carrier [40]. A positive identification of a carrier should be reliable. If the mutation in the proband is known, analysis of the DNA for heterozygosity in relatives is precise.

Prenatal diagnosis may be carried out by assay of ^{35}S-mucopolysaccharide accumulation or the activity of iduronidase [41]. In the 35S assay, normal amniocytes behave just like fibroblasts, but iduronidase activity is much lower in amniocytes than in fibroblasts, and this could cause difficulties in distinguishing a heterozygote from an affected fetus. Prenatal diagnosis has also been accomplished by assay of iduronidase in chorionic villi [42], but activity is normally so very low in this material that great care is required.

The gene for iduronidase consists of 14 exons and approximately 19 kb. A large 13 kb intron separates the second and third exons [7]. There is a canine model of MPS I, and the canine gene for iduronidase has the same structure as the human gene [43]. The mutation in the model is a G-to-A transition in the donor splice site of intron 1, which leads to retention of this large intron in the RNA and premature termination of the intron-exon junction. A locus D4S111 linked to Huntington disease on chromosome 4 has turned out to be the iduronidase gene [44].

The mutations that account for more than 50 percent of the alleles in populations of European origin change a tryptophan at position 402 and a glutamine at 70 to stop-codons; both yield no detectable functional protein [8, 9, 10]. Premature termination is also present at a deletion/insertion in exon 6 for which a Libyan Jewish patient with Hurler disease was homozygous [11]. Stop-codons resulting from changes of tyrosine 64 and glutamine 310 were found in Arab patients [12], as well as a threonine-to-proline change at 366 and a glycine-to-arginine change at 409. Among Japanese, there are two common mutations, a 5bp insertion between nts704 and 705, and R89Q which is uncommonly seen in Caucasians [45]. Homozygotes for all these mutations except the Japanese missense mutation and compounds of any two of the others have a severe Hurler phenotype. Homozygosity for the first of these Japanese mutations conveyed a severe phenotype. Splice-site mutations and deletions have also been observed [46]. In addition to the considerable mutational heterogeneity among MPS I patients, there are many polymorphic alleles consistent with common haplotype structure [47]. In homozygous setting null mutations and coding disruptions lead to the severe Hurler phenotype [47]; missense mutations are found that are individually characteristic of the H, HS, or S phenotypes.

Newborn screening is feasible. Chamoles *et al.* [48] demonstrated that the enzyme is stable in dried blood spots, and an assay using tandem mass spectrometry has been developed [49].

High-throughput mass spectrometry has been developed to screen newborns for lysosomal storage disorder [50]. It is currently feasible to diagnose Pompe, Fabry, Gaucher, Krabbe, and Niemann-Pick A/B diseases, as well as MPS I, by tandem mass spectrometry of dried blood spots. The methodology was validated in established patients and controls.

TREATMENT

The discovery of the MPS correcting factor capable of correcting the defective glycosaminoglycan catabolism in cultured cells raised the hope that these diseases might be

treatable by transplantation or enzyme replacement therapy. The availability of animal models and recombinant enzyme with the mannose-6-phosphate recognition signal [51] as well as successes in the clinical management of Gaucher disease provided hope for successful enzyme replacement therapy. Recombinant iduronidase prepared in hamster cells administered to homozygous animals led to major improvement in storage in liver, spleen and kidney, but no improvement in brain, heart valves or cornea [51].

Enzyme replacement with human recombinant α-L-iduronidase has been reported [52, 53] in 45 patients with MPS I. Patients were selected with Hurler-Scheie or Scheie phenotypes and given enzyme intravenously weekly for as long as 62 weeks. Hepatosplenomegaly decreased significantly in all patients. Liver size was normal in eight patients by 26 weeks. Growth in height and weight increased in prepubertal patients. Improvements were also notable in the urinary excretion of glycosaminoglycans, as well as in joint mobility, respiratory function (forced vital capacity) [52, 53], and ambulation (6-minute walk test). The enzyme has now been approved by the US Food and Drug Administration (FDA) (in 2003) and is marketed as Aldurazyme (BioMarin Genzyme). Corneal clouding does not change; valvular disease seems to be unaltered. There is little likelihood that this approach would affect the brain; trials are underway to treat with intrathecal enzyme [54]. Enzyme replacement therapy has been used for a sufficient number of years in the treatment of Hurler disease to establish that it is efficacious in reducing biochemical characteristics of the disease such as urinary glycosaminoglycan excretion and improved function capacity, as well as liver size [55].

Engineering of α-iduronidase by fusion to a receptor-binding peptide from apolipoprotein E (apoE) was carried out to bind to LDL receptors on the blood brain barrier [56]. In mice with MPS I this treatment yielded 2–3 percent of normal iduronidase activity in brain and normal levels of glycosaminoglycan in brain.

Bone marrow transplantation [57] was followed by arrest or reversal of many of the peripheral features of the disease. It did not seem likely that this would appreciably affect the central nervous system, but longer-term follow up of the results of bone marrow transplantation in Hurler disease [58] have documented resolution of hydrocephalus and, in four patients with normal IQs before the procedure, maintenance of intelligence for two to seven years post-transplantation. It appears clear that if performed early enough bone marrow transplantation will preserve cerebral function.

Magnetic resonance spectroscopy indicated high ratios of presumptive MPS to creatinine that did not fall after bone marrow transplantation [59]. Bone marrow transplantation appears not to improve the skeletal or ocular manifestations of the disease. In patients without a compatible donor, unrelated umbilical cord blood transplantation may be an option. It is possible that transplantation and enzyme replacement therapy may be complementary. Outcomes of allogeneic transplantation have been reported [60] in 258 children with HS. Overall survival was 74 percent at five years.

In patients with stop codon mutations, stop codon read through (SCRT) [61] is a potential method to achieve enhanced enzyme activities. Chloramphenicol has been used as a peptide transferase inhibitor to induce read through, it does cross the blood-brain barrier. It enhances iduronidase activity [61] in fibroblasts derived from patients.

Supportive management includes shunting for hydrocephalus, surgical decompression for carpal tunnel syndrome, tonsillectomy and adenoidectomy for airway obstruction, and otitis media, hearing aids and visual aids. Inguinal hernias should be repaired. Cardiac valvular surgery may be indicated.

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77

Scheie and Hurler–Scheie diseases/ mucopolysaccharidosis IS and IHS/α-iduronidase deficiency

MAJOR PHENOTYPIC EXPRESSION

Scheie: stiffness of joints, corneal clouding, disease of the aortic valve, dystosis multiplex, normal intelligence. Hurler–Scheie: intermediate between Hurler and Scheie.

INTRODUCTION

In 1962, Scheie, Hamprick, and Barness [1] described the phenotype as a "forme fruste of Hurler's disease". This was prescient, as it turned out that the phenotypes are allelic, both resulting from deficiency of the enzyme α-iduronidase (see Figure 77.1). It was the delineation of corrective factors by Fratantoni, Hall, and Neufeld [2] that led to the clear recognition that the Hurler and Scheie genes were allelic, because sulfate accumulation in Hurler fibroblasts was not cross-corrected by Scheie cells, and vice versa [3], and Hurler–Scheie corrective factor was identified as α-L-iduronidase [4]. Both Hurler and Scheie fibroblasts contained no demonstrable α-iduronidase activity against substrate phenyl-L-iduronide [5, 6].

The intermediate Hurler–Scheie phenotype was first named on clinical grounds by McKusick [7], who postulated that Hurler IH and Scheie IH phenotypes represented homozygosity for one or the other allele and predicted that there would be compounds which expressed an intermediate phenotype that he called IH-S [7]. Actually, it turns out that some of the intermediate phenotypes represent homozygosity for some specific mutations [8]. The cloning of the iduronidase gene on chromosome 4 [9]

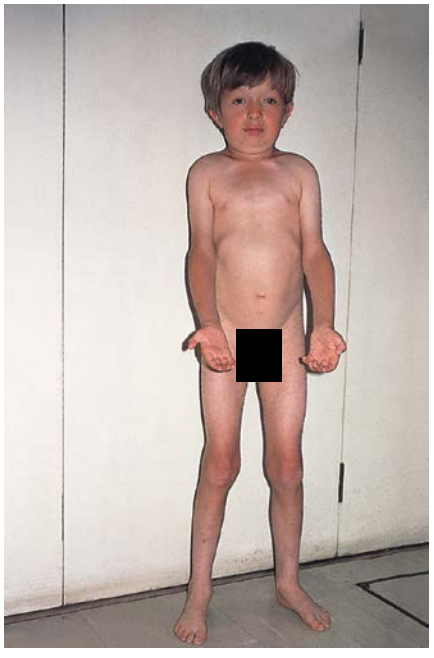

Figure 77.1 A seven-year-old boy with Scheie disease illustrates the early claw hand deformities and gene valgum. (Illustration was kindly provided by Dr Philip Benson.)

made it clear that there are many mutations and more to be discovered. Compounds are found even within each of the phenotypes, as are homozygotes. Among the latter with the H-S phenotype are P533R, which is the most common, and particularly common in Morocco [10] where it is the only mucopolysaccharidosis (MPS) I mutation found to date, and A327P found in Italy and Brazil [11, 12]. The Scheie phenotype was found in Brazil [11] and commonly in Japan [13] with homozygosity for R89Q.

CLINICAL ABNORMALITIES

Scheie disease

The Scheie phenotype has been of particular interest to ophthalmologists, because patients live long enough for the severe corneal clouding to affect vision. It is most dense on the periphery. The patient may first be aware early in the second decade, but it is diagnosable by slit lamp very early. There may also be pigmentary degeneration of the retina. Some patients develop glaucoma. Visual impairment may progress to blindness.

Abnormalities of the joints may be evident early in childhood (Figure 77.1), at least by the age of five years. Joints are stiff and angulated [14]. The claw hand may be identical to that of Hurler disease. Genu valgum is present early. There may be pes cavus and a stiff painful foot. Carpal tunnel syndrome is a common complication due to entrapment of the median nerve [15]. Distal interphalangeal acute angulation gives a trigger-finger appearance [15]. Degenerative arthritis of the hip has been reported [16] along with large femoral cysts and pathologic fracture, but this appears to be rare. Stature is normal.

Facial features may be somewhat coarse but are not often recognizable as those of MPS. Hypertrichosis is common, and so are inguinal hernias. Some patients develop deafness, and it can be progressive.

Life expectancy may be normal except in those that develop cardiac disease [17]. Aortic stenosis or regurgitation may be evident even early, but, as deposits of mucopolysaccharide increase on the valves and chordae tendinae, disability may develop [18–20]. Sleep apnea was reported in two brothers, 18 and 35 years of age, which was relieved by tracheostomy [21].

Neurologic manifestations are uncommon, but myelopathy has been reported as a consequence of cervical cord compression from thickened dura, the so-called pachymeningitis cervicalis [22, 23]. This problem is more common in the H-S variants. Attenuated forms of this and Hurler–Scheie disease have proved especially difficult to diagnose. Early symptoms of joint pain and stiffness along with inflammatory features have led to referral to rheumatologists or dermatologist. In Scheie disease, the diagnosis is often not considered until adulthood [24].

Hurler–Scheie disease

The clinical features of these patients are intermediate between those of the Hurler and Scheie phenotypes (Figures 77.2–77.11). Features may be coarse (Figure 77.2) or not increasing especially with time (Figure 77.11); an adult patient may have very grotesque features, having lived so much longer than a Hurler patient, and consequently had time to accumulate large amounts of mucopolysaccharide. Some patients have micrognathism, and this may contribute to a distinctive facial appearance [25]. Intellectual function may be normal; some have impaired mental development. Survival to adulthood is common. Pregnancy has been reported [26]. Stature is short.

Figure 77.2 CL: A 13-year-old girl with Hurler–Scheie disease. Her face in repose showed clear evidence of mucopolysaccharide storage especially about the lips and nose. Corneas were slightly cloudy.

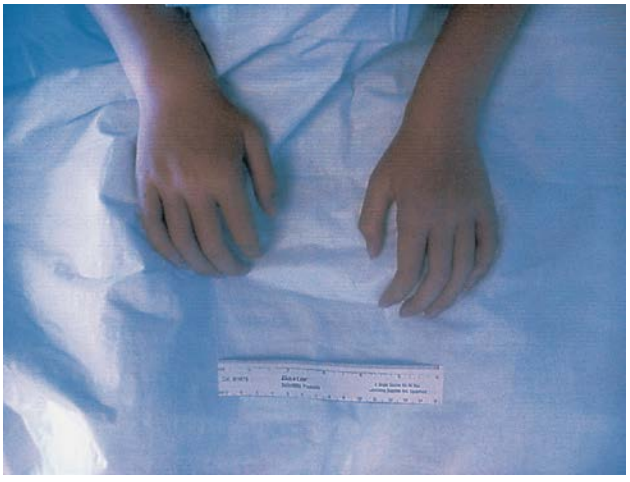

Figure 77.3 CL: She had the claw hand with considerable limitation of motion. There were also contractures at the shoulders and elbows, and she could not raise her hands above her head.

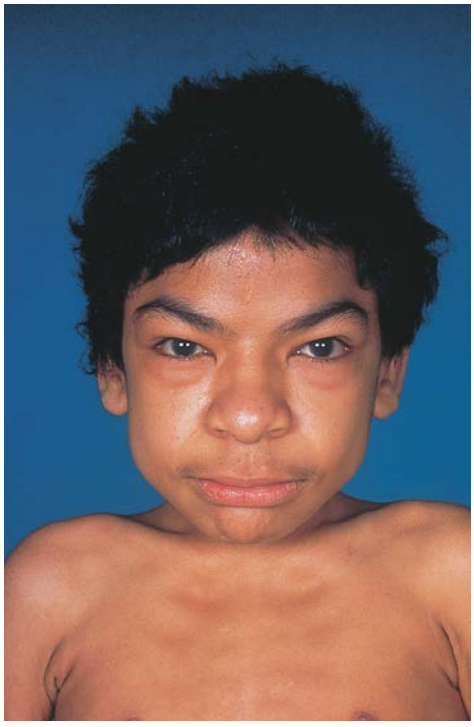

Figure 77.4 HY: A 16-year-old Saudi boy with α-iduronidase deficiency and the clinical phenotype of the Hurler–Scheie syndrome. Formal psychometric testing revealed the intelligence to be normal. He was short; height was 129 cm, and he had coarse facial features, including a large nose and thick lips and hirsutism.

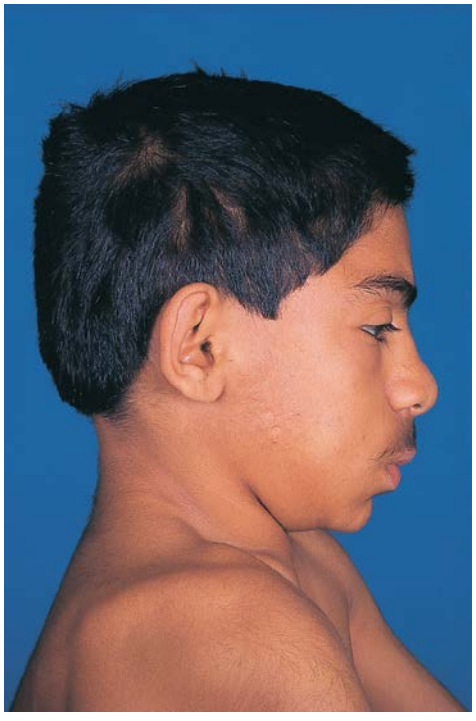

Figure 77.5 HY: There was some micrognathism.

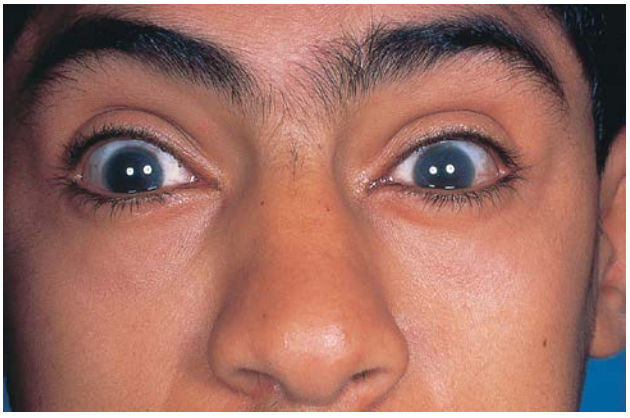

Figure 77.6 HY: There was bilateral clouding of the cornea that ultimately led to corneal transplantation.

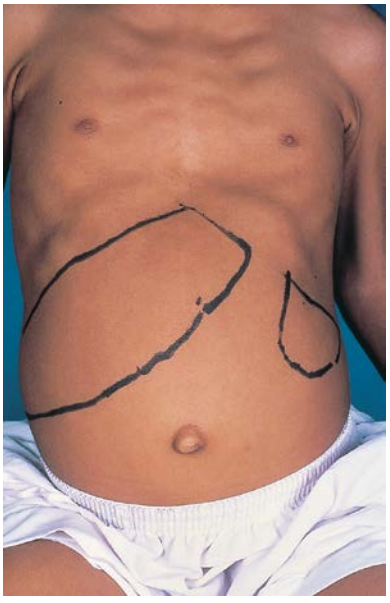

Figure 77.7 HY: Liver and spleen were enlarged as outlined.

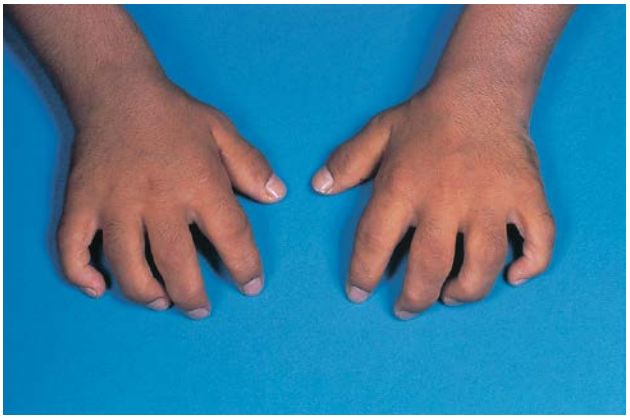

Figure 77.8 HY: The hands were broad, short, and flexed, and had a Hurler appearance. There was limitation of joint motion, which was progressive, and he had chronic joint pains.

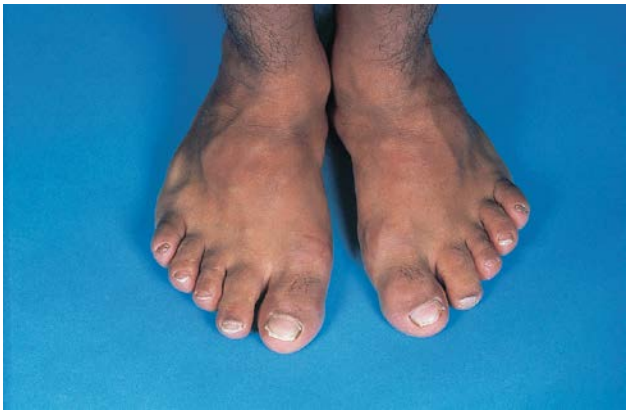

Figure 77.9 HY: The feet were also short and broad.

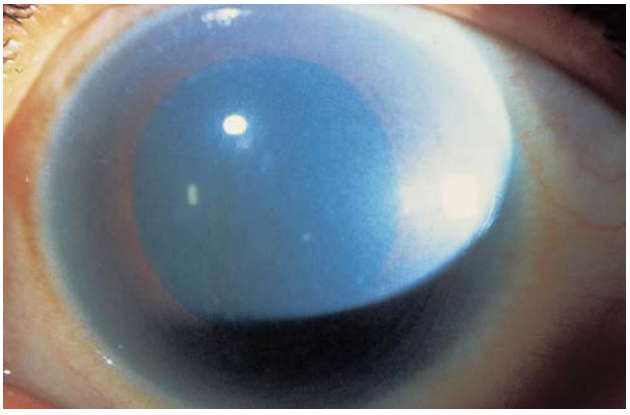

Figure 77.10 AMA: The cornea was quite cloudy and he had glaucoma. Two sisters had Hurler–Scheie disease and all had deficient activity of α-iduronidase. Parents were consanguineous.

Clouding of the cornea is a regular feature of this disorder. In fact, only one patient has been reported with iduronidase deficiency in whom the corneas remained clear (to 14 years at the time of report) [27].

Hernias, stiffness of the joints, and the classic claw hand (Figure 77.8) are seen uniformly; so is hepatosplenomegaly (Figure 77.7). Lesions of the cardiac valves may cause cardiac failure and death. Myelopathy from cord compression is a frequent complication in this condition, as is hydrocephalus resulting from mucopolysaccharide deposition in the meninges [28]. Increased intracranial pressure led to muscle weakness and spasticity attributed to obstruction of the basilar cisterns [28]. That patient first presented at 25 years of age with paranoia. Psychosis has also been observed in the Scheie syndrome. Another patient had marked destruction of the sella and the cribriform plate and spinal fluid rhinorrhea, as well as blindness from pressure on the optic nerves.

The pathologic appearance of the Hurler–Scheie and Scheie disease is that of widespread deposition of mucopolysaccharide [25, 28]. The thickened dura may contain foamy macrophages and increased quantities of

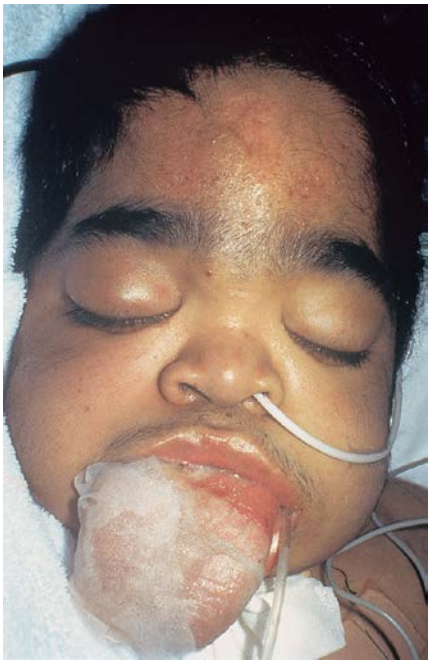

Figure 77.11 A 22-year-old with Hurler–Scheie syndrome. Facial features were classic. The enormous tongue had led to respiratory obstruction and tracheotomy. The patient was in intensive care. Liver and spleen were greatly enlarged and he had typical trident hand.

collagen. In MPS IS, cortical neurons have been reported as normal, while somatic cells are no different than those in MPS IH. In MPS IHS, changes in cortical neurons are less frequent than those seen in the anterior horn cells of the cord, in which there are typical concentric lamellar inclusions.

GENETICS AND PATHOGENESIS

The Scheie and Hurler–Scheie phenotypes are inherited in autosomal recessive fashion. The fundamental defect is in the α-L-iduronidase [5]. In general, it has not been possible to distinguish the Scheie from more severely affected individuals on the basis of residual enzyme in the usual assays with artificial substrates [29, 30]. Some residual activity has been reported in a radioactive disaccharide assay [31], and others have found some activity in fibroblasts of Scheie patients when desulfated heparan was the substrate [32]. The Scheie disease has been estimated to occur at a frequency of one to 500,000 births in British Columbia [33]. The overall prevalence of MPS I is 1 per 100,000 births [34].

It became clear that patients with the Hurler–Scheie phenotype have resulted from consanguineous matings [35], indicating that the phenotype may result from homozygosity for single mutant alleles rather than compounds of Hurler and Scheie alleles. Cell hybridization studies of all three phenotypes have led to failure of complementation [36]. These issues have been clarified by

the definition of mutations. One patient with the Scheie phenotype has been found to have an allele with a G-to-A transition in intron 5 which creates a new acceptor splice site without losing the original site; thus, some normal enzyme is produced [37, 38], as demonstrated by enzyme assay of fibroblasts of the patient [39]. Compound heterozygosity for R89Q, which causes a mild phenotype when homozygous, and 704ins5, which causes a severe phenotype, produced an intermediate pattern of disease in Japanese patients. As little as 0.4 percent of normal enzyme is enough to lead to a mild phenotype [40]. Enzyme assay is not sufficient for carrier detection because normal individuals and carriers overlap [41].

In a series of 85 MPS I families [42] none of the Scheie patients were mentally retarded. In a 34-year-old patient with Scheie disease [43] the mutations were c.1190-1 del G/N and c.1708G>C/N [44].

TREATMENT

The supportive management and enzyme replacement set out in Chapter 76 is particularly appropriate for Scheie and Hurler–Scheie patients. Hematologic stem cell transplantation, especially if performed before two years of age has resulted in rescue of neurocognition [45]. In 45 patients with attenuated disease recombinant laronidase had decreased excretion of glycosaminoglycan and decreased hepatic volume, improved shoulder flexion and decrease in sleep apnea. Corneal transplantation has been successful [46, 47]. Aggressive surgical treatment of glaucoma and carpal tunnel syndrome is also indicated. Cardiac valve replacement has also been successful in both IS and IH/IS patients [18, 19]. Mitral valve replacement and resection of a large left atrial appendage was successful in a patient with Hurler–Scheie syndrome [48]. Hydrocephalus requires shunting, and cervical cord decompression may be required.

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78

Hunter disease/mucopolysaccharidosis type II/ iduronate sulfatase deficiency

MAJOR PHENOTYPIC EXPRESSION

Coarse features, stiff joints, short stature, impaired mental development, hepatosplenomegaly, cardiomegaly, nodular or thickened skin lesions especially over the scapular area, dysostosis multiplex, accumulation of dermatan sulfate and heparan sulfate, and defective activity of iduronate sulfatase.

INTRODUCTION

In 1917, Hunter [1] described two brothers with what is now known as mucopolysaccharidosis (MPS) type II. Patients with Hunter disease have clinical features similar to those of Hurler disease, although usually they are less severely affected. Patients have been classified clinically into mild and severe forms, although the two cannot be distinguished on the basis of enzyme activity. The advent of molecular analysis and extensive heterogeneity may make this classification obsolete. Patients with this disease were found, by Dorfman and Matalon [2] and by Muir [3], to excrete dermatan sulfate and heparan sulfate just like those with Hurler disease. It was in studies of Hunter and Hurler cells that Fratantoni, Hall, and Neufeld [4] first found the correction factors from each that corrected the defective excess accumulation of sulfate in the other; thus Hurler cells could correct Hunter cells and vice versa, and the Hunter corrective factor would correct Hurler and other MPS cells, but not Hunter cells [5]. The Hunter factor was identified as iduronate sulfatase [6] (Figure 78.1), the enzyme that catalyzes the release of sulfate from the iduronate sulfate moieties of dermatan and heparan sulfates. This is the site of the molecular defect in Hunter disease. Thus, the defect in MPS II is in the first step in the enzymatic breakdown of these mucopolysaccharides.

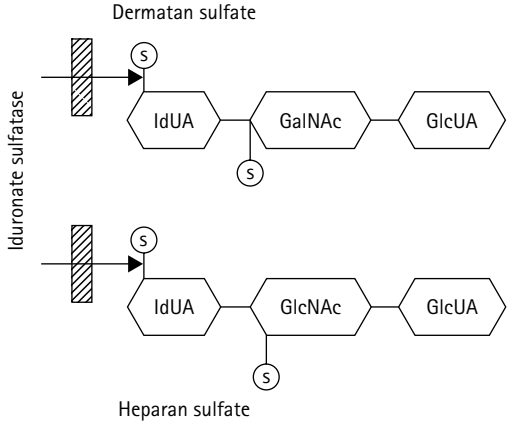

Figure 78.1 Iduronate sulfatase, the site of the enzyme defect in Hunter disease. Both dermatan sulfate and heparan sulfate accumulate when activity is defective.

The gene for iduronate sulfatase has been identified [7] and mapped to the X chromosome at position q28 [8–10]. A number of gross alterations in the gene have been found [7, 11], as well as point mutations, especially at CpG dinucleotides [12, 13].

CLINICAL ABNORMALITIES

Patients with Hunter disease present a broad spectrum of clinical activity; all of them have quite similar reduction in enzyme activity. Nevertheless, the disease has generally

been subdivided into two groups, of severe and mild phenotypes, respectively [14, 15].

Patients with the severe form may appear identical to those with Hurler disease (Figures 78.2 and 78.3) except for the absence of cloudy corneas, but behavior is usually quite different. Progression may be slower than in Hurler disease and the apparent onset may be later, often about two to four years of age. In the mild form (Figure 78.4), mental development may be normal, and lifespan may be long as in Scheie disease.

Hunter disease is distinguished from all other mucopolysaccharidoses by the presence of nodular or pebbly skin lesions (Figure 78.5), most characteristically over the scapular area, the upper arms or the lateral aspects of the thighs. The skin lesions are sometimes ivory in color. These lesions are not seen in any mucopolysaccharidosis except Hunter disease [16].

As in other forms of mucopolysaccharidosis, chronic respiratory symptoms, rhinorrhea or stertorous breathing, or frequent upper respiratory infections and otitis media may be the earliest manifestations of disease. Presentation for hernia repair may be even earlier. Both inguinal and umbilical hernias are common. Mental development

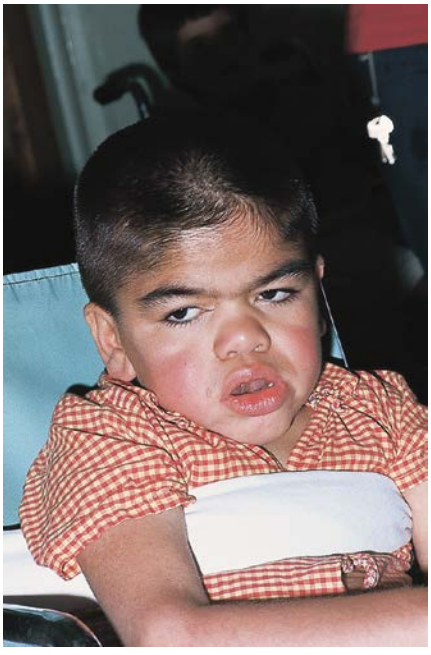

Figure 78.2 CT: A patient with Hunter syndrome. In this most severe form of the disease, the features are quite coarse, the lips very thick, hirsutism prominent, and the hands claw-like, as in Hurler disease. The corneas were clear.

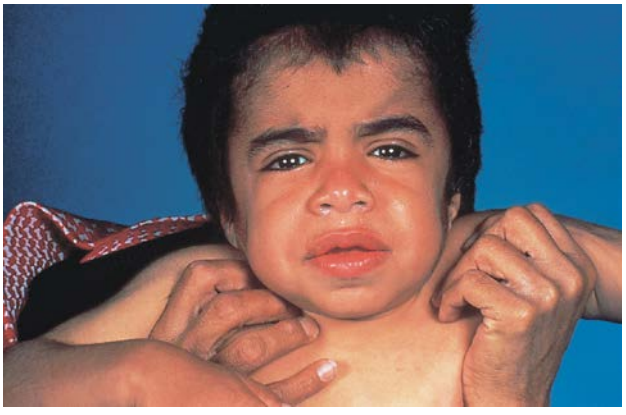

Figure 78.3 ADFS: An eight-year-old boy with Hunter disease. The facial features were quite coarse, the hairline low, and the eyebrows abundant, and the lips were very full. Iduronate sulfatase activity was absent.

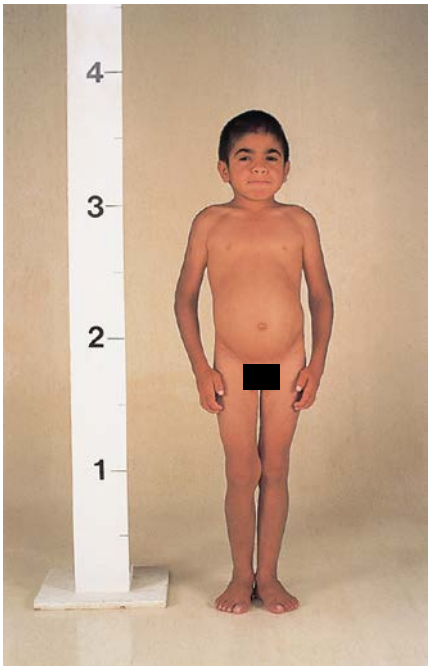

Figure 78.4 AMAQ: A six-year-old boy with a milder expression form of Hunter disease. The hairline was low and the eyebrows bushy. The increased subcutaneous tissue was clearly evident in the anteverted nose, but features were less coarse than the patient in Figure 78.2. Limitation of joint motion was visible in the flexed elbows.

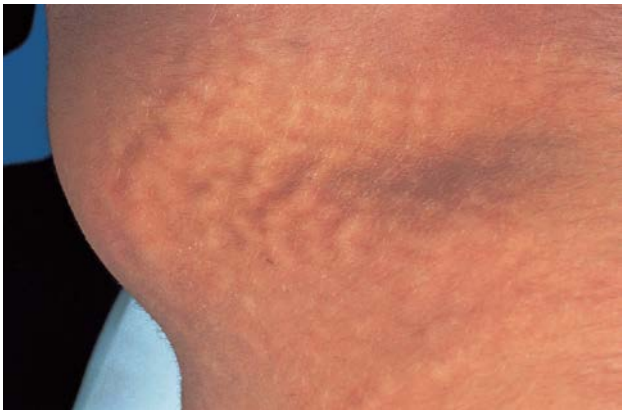

Figure 78.5 ADFS: The nodular or pebbly skin lesions constitute a cutaneous marker for the Hurler disease.

usually continues until at least two years of age. Hearing loss is common and progressive [17].

Patients with the severe form of Hunter disease have the characteristic coarse features of mucopolysaccharidosis (Figures 78.2, 78.3, 78.6, and 78.7) [18]. The nose is flat, the nasal bridge depressed. The lips are thickened, the gums hypertrophic, and the tongue is large. Patients are generally hirsute and have low hairlines (see Figures 78.2 and 78.3). The superciliary ridges become very prominent. The head may appear disproportionately large. Stature is short, but this may not be as pronounced as in Hurler disease. Joints are stiff and mobility may become limited, or there may be contractures. Hearing loss is common; it may not be severe [19], but it tends to be progressive. The hands are broad and the fingers stubby. The claw-hand appearance (Figures 78.6 and 78.7) may be indistinguishable from that of Hurler patients. Patients tend to develop high coloration. The liver and spleen are large and hard.

The important negative finding in the Hunter syndrome – the absence of a cloudy cornea [20] – distinguishes it from Hurler syndrome. These patients may develop a rounded kyphosis and occasionally there is a severe kyphosis [21]. Corneal clouding may even be detected very late in the most severe forms of Hunter syndrome, but usually only with a slit lamp [22].

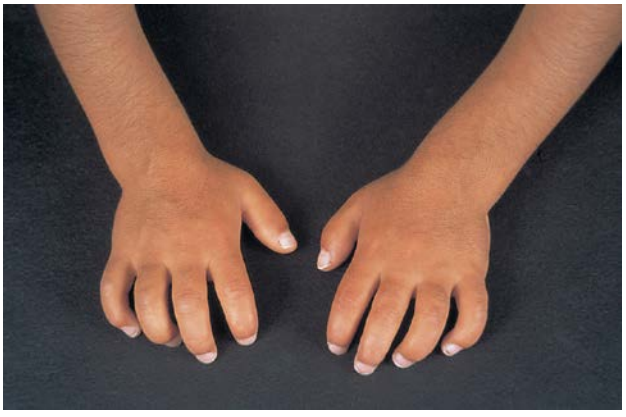

Figure 78.6 AMAQ: The claw hands were typical for mucopolysaccharidosis.

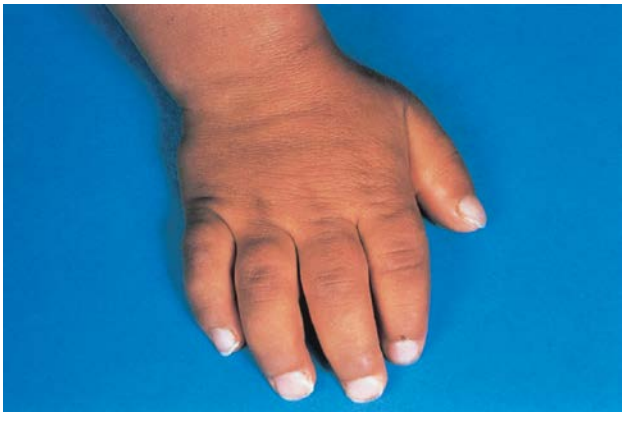

Figure 78.7 The hand of ADFS was also typical.

The voice is hoarse. Diarrhea may be a chronic problem; it may result from infiltration of the autonomic innervation of the intestine [23]. Retinitis pigmentosa may occur in this condition and retinal degeneration may cause blindness. Glaucoma may be a problem. Papilledema may be seen [24]; this is probably a consequence of pachymeningeal thickening, which may also lead to neurologic defects including quadriplegia from pressure on the cord [25]. It may also result in hydrocephalus [26]. Cerebral atrophy, which may also lead to ventricular enlargement, is seen regularly on computed tomography (CT) scan or magnetic resonance imaging (MRI) in severe Hunter disease [27–29], and there may be defective reabsorption of cerebrospinal fluid. Intracranial pressure may be increased.

Mental deterioration is progressive, but usually occurs at a slower rate than in Hurler disease. Rarely mental deterioration may be profound early in life.

The behavior of a patient with severe Hunter disease is often characteristic [19] and contrasts sharply with the sweet disposition of the Hurler patient. From two to six years of age, the Hunter patient may develop primitive, uncontrolled activity in which he throws toys and seems to enjoy self-created noise. He is hyperkinetic. Rough, aggressive play may be dangerous to pets or younger siblings. These patients are often stubborn, fearless, and unresponsive to discipline. Eating habits may be unusual, and pica is common. One patient developed lead poisoning. The management of such a child is difficult and admission to an institution is common.

Obstructive airway disease may result from infiltration of the vocal cords or trachea, or a large tongue. Tracheostomy may be necessary. Cardiac complications, such as congestive failure, result from valvular or myocardial infiltration. Coronary insufficiency may result from infiltration in the vessels. Thickened valves may be demonstrated by echocardiography. Some have pulmonary hypertension.

Most patients deteriorate progressively after five or six years of age. Physical activity decreases; gait may become unsteady; and speech deteriorates and ultimately is lost. Difficulty in ingesting solid food is progressive, and there is loss of weight. Respiratory infections become more frequent and more severe and may be the cause of death. Generalized seizures may occur in the final months of life. Death usually occurs by 15 years of age from respiratory or cardiac disease. In a series of 31 patients, mean age of death was at 13 years in those with severe disease and 23 years in those with a mild phenotype [30]. Disordered breathing during sleep has been observed [32]. Respiratory failure was the most common cause of death (56%) followed by cardiac failure (18%). Patients with the milder forms of the disease may survive well into their sixties or beyond [33]. Intelligence may be preserved. Features may appear normal in childhood, or may be mildly coarse (Figure 78.4), but with time the appearance becomes increasingly recognizable. Joint stiffness may be an increasing problem,

and patients may develop osteoarthritis. Carpal tunnel syndrome is common. Extensive Mongolian spots over the buttocks and lumbar sacrum region were observed [34].

Hearing loss is regularly observed. Retinal dysfunction may be documented by electroretinography [35]. Chronic papilledema has been reported in the absence of increased intracranial pressure [36, 37]. Hydrocephalus appears to be rare in the mild forms of Hunter disease [28]. Arachnoid cysts have been observed [38]. Spinal stenosis, especially cervical may cause cord compression [39].

Survival for as long as 87 years has been observed [32], but death may occur in the second decade, even in the mild phenotypes. The cause may be cardiac disease, pulmonary infection, or airway obstruction. Sudden death after complete atrioventricular block has been reported [40].

The roentgenographic picture of all forms of the disease is that of dysostosis multiplex. The features may be quite similar to those of Hurler disease, but they tend to be less dramatic (Figures 78.8–78.10). External thickening of the cortices of the bones may be seen early. With increasing age, the cortical walls become thinner as the marrow cavities expand [41]. The skull is large and the sella shoe shaped. The lower ribs are broad and spatulate. There is hypoplasia of the vertebrae and beaking of L2 (Figure 78.9). Large radiolucent areas surrounding the unerupted teeth represent dentigenous cysts, not accumulation of mucopolysaccharide [42]. Smaller lucent lesions may be collections of collagen. In attenuated patients, volume of the corpus callosum was decreased [43]. The volume of white matter increased less than in controls.

Fundamental to the clinical phenotype is the excessive intracellular accumulation of acid mucopolysaccharides. Large vacuolated cells containing metachromatic cytoplasmic material are present on histologic examination of many tissues. In the scapular nodules, there is extracellular accumulation of metachromatic material [44]. Dermatan sulfate and heparan sulfate [2, 14] are excreted in the urine in large and approximately equal amounts. Cultured fibroblasts show metachromatic staining and contain large amounts of mucopolysaccharide [2]. Hunter cells accumulate labeled sulfate in a typical mucopolysaccharidosis pattern.

GENETICS AND PATHOGENESIS

Hunter disease is inherited as an X-linked recessive trait. Patients with specific mild or severe phenotypes closely resemble other affected members of an individual family. The incidence of the disease has approximated one in 100,000 male births in Great Britain, the Netherlands, Germany, and British Columbia [45], and one in 36,000 in Israel [46,47,48]. It is more common in Asians than other types of MPS. The disease has been recognized in a small number of female individuals. One had an X:5 autosome translocation in which the breakpoint at the gene locus caused the disease, because the normal X was inactivated [49, 50]. Others represented nonrandom inactivation of a normal X chromosome, including one of a pair of nonidentical twins [50–53].

The molecular defect is in the enzyme iduronate sulfatase (Figure 78.1) [6, 54, 55]. The enzyme has been purified from human liver [56], placenta [57], and plasma [58]. The human cDNA codes for a polypeptide of 550 amino acids [10]. The enzyme [59], which removes the sulfate from the 2-position of iduronic acid, is essential for the sequential degradation of heparan sulfate, which contains many sulfated iduronic acid residues, and dermatan sulfate, which contains a

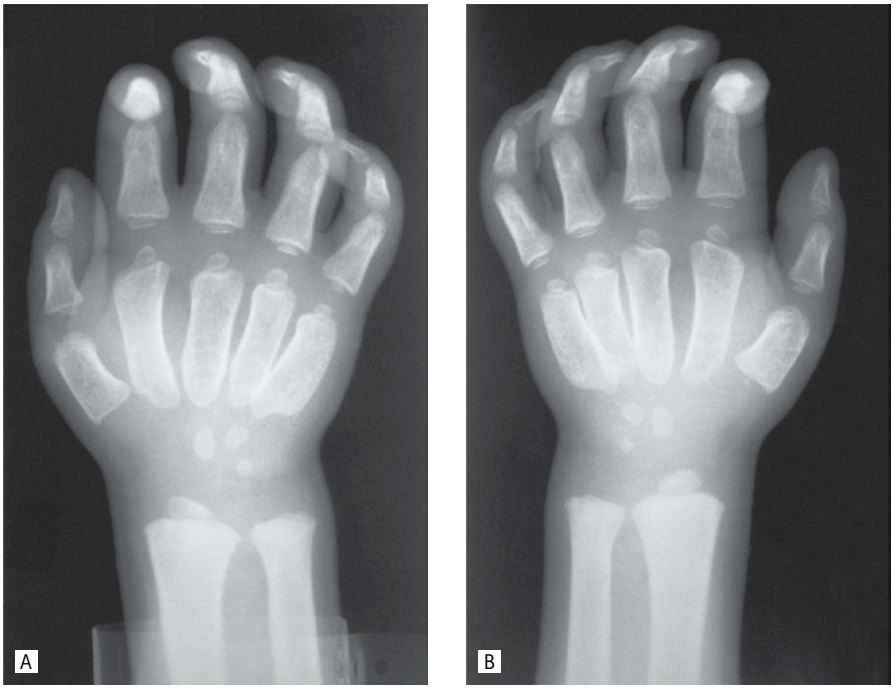

Figure 78.8 AMAQ: Roentgenograms of the hands illustrate the broadened phalanges and metacarpals, as well as the fixed flexion deformities. The proximal ends of the metacarpals are tapered.

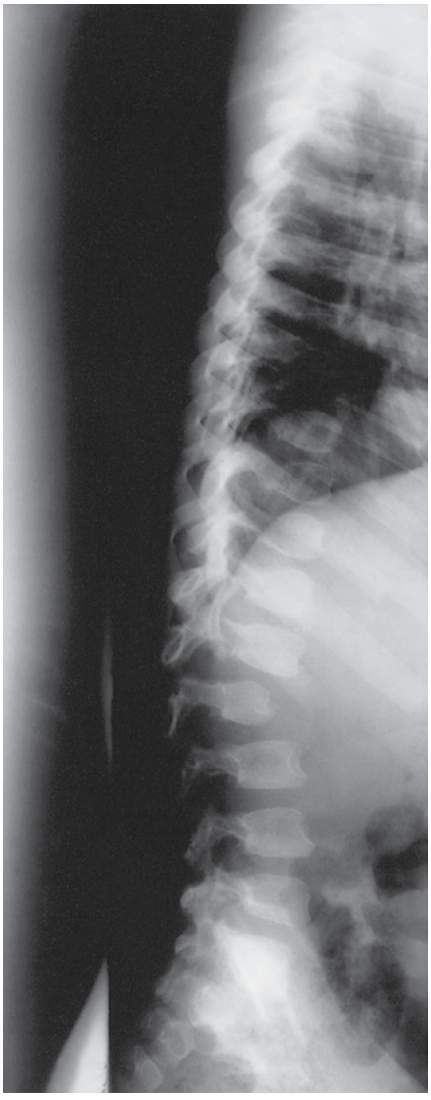

Figure 78.9 AMAQ: The L2 vertebra was beaked and displaced posteriorly.

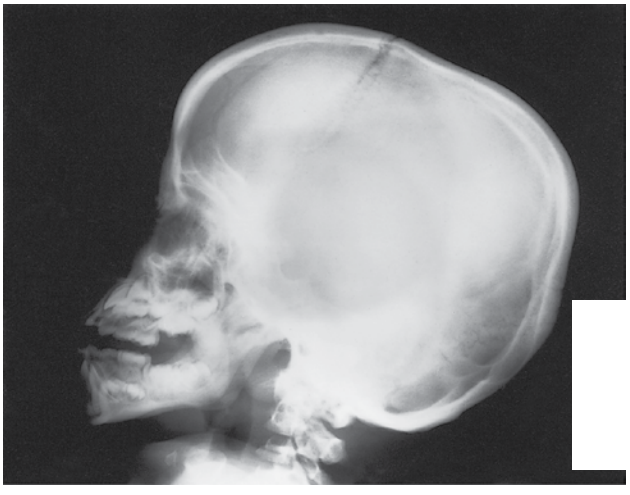

Figure 78.10 The skull of AMAQ illustrates the thickened cranial vault.

smaller number of such residues. The failure to degrade even a single sulfated uronic acid leads to the accumulation of the glycosaminoglycan. Enzymatic analysis for the activity of iduronate sulfatase fails to distinguish among the mild and severe clinical phenotypes; in all of them there is virtually complete absence of enzyme activity.

β-Galactosidase activity is diminished in the skin and other tissues of patients with the disease [60]. This abnormality, which also occurs in Hurler disease, is secondary to the primary defect, but it could relate to the accumulation of ganglioside and other lipids found in the brain of patients.

The diagnosis, once suspected clinically, has been confirmed by the quantitative assay of the excretion of total glycosaminoglycans in the urine, but the specific diagnosis depends on the assay for iduronate sulfatase, which can be carried out on serum, cells, or tissues [61]. None of the screening tests for mucopolysaccharide in the urine is completely reliable.

Heterozygous female carriers of the Hunter gene have been recognized by cloning of fibroblasts followed by assessment of the accumulation of ^{35}S-mucopolysaccharide [62] or assay of iduronate sulfatase in individual hair roots [63, 64]. Two clonal populations, one normal and the other abnormal, have been demonstrated using both these techniques consistent with Lyon hypothesis. Demonstration of a major deletion in the gene provides a highly accurate and less demanding approach to carrier detection.

Prenatal diagnosis was initially carried out successfully by using sulfate incorporation in cultured amniocytes [65]. It is now done by assay of the enzyme in amniocytes. It is recommended that the early information obtained from the fluid always be confirmed by assay of the cultured cells. Prenatal diagnosis has also been accomplished by assay of the enzyme in chorionic villus homogenates [66]. In the case of female fetuses, very low levels of enzyme may be found in either amniocytes or chorionic villus cells; therefore, it is important that karyotyping be carried out in all instances. In families in which the mutation is known, molecular methods are of choice for prenatal diagnosis and heterozygote detection.

The gene for iduronate sulfatase is very large. It contains nine exons over 24 kb [67, 68]. Complete or partial deletions of the gene were identified in patients with the severe phenotype [57, 69]. Among this population of patients with severe disease, deletions or rearrangements visible in Southern blots occur in about 20 percent [7, 11, 70–72]. Identical changes have been found in unrelated patients [66]. In addition, a number of missense and nonsense 7 mutations have been identified [11–13, 68, 71–76]. In 65 Japanese families with MPS II, there were 16 mutations [77]. Those with attenuated phenotype generally had missense mutations. Structural alterations in the gene were found in the severe phenotype. Approximately half of the single-base substitutions have occurred at CpG dinucleotides, suggesting independent origin in different families [78]. Codon R468 when changed to W led to mild disease in

a patient in the United States [13] and severe disease in a Japanese [72], typifying the problem of genotype–phenotype correlation; it was also changed to Q, L, and G in severely affected patients [13, 79, 80]. New mutation has been found to occur more frequently in the genesis of the heterozygous carrier than of the affected male [71, 77].

TREATMENT

Specific treatment for this disease continues to be explored. Enzyme replacement therapy with human idursulfatase produced by recombinant DNA technology has been administered intravenously [81–84]. Urinary levels of GAG were reduced. Splenic volume was decreased [81]. Mental decline has not been arrested. Treatment was approved by the FDA in 2006. In a double-blind placebo-controlled trial [82] urinary GA6 were reduced in two weeks and liver and spleen value decreased by 24 weeks. In a trial, forced vital capacity and the 6-minute walk test were significantly reported [83]. Currently idursulfase is given in weekly, 3-hour infusions at 0.5 mg/kg. Bone marrow transplantation has been performed in this disease [85]. It may improve respiratory problems or the size of liver and spleen. It does not improve cerebral function.

A variety of supportive measures are useful, especially in the milder forms of the disease, in which longer survival is associated with some painful complications. Shunting is important in the management of hydrocephalus. Hearing aids may aid in deafness. Physiotherapy is useful for the joint stiffness and the avoidance of contractures. Surgical decompression is carried out for carpal tunnel syndrome. Cardiac valvular status should be monitored by echocardiography. Tracheostomy or nasal continuous positive airway pressure may alleviate obstructive airway disease.

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79

Sanfilippo disease/mucopolysaccharidosis type III

MAJOR PHENOTYPIC EXPRESSION

Mental deterioration, mild skeletal dysostosis multiplex, and urinary excretion of heparan sulfate. The Sanfilippo disease type A is due to a deficiency of heparan-N-sulfatase, type B to a deficiency of α-N-acetylglucosaminidase, type C to N-acetyl-CoA: α-glucosaminide acetyl transferase, and type D to N-acetylglucosamine-6-sulfatase.

INTRODUCTION

This disorder was first described by Harris [1] in 1961 with the report of a six-year-old girl with mildly impaired mental development who had hepatosplenomegaly and a normal skeletal survey and excreted large amounts of heparan sulfate in the urine. Sanfilippo and colleagues [2, 3] in 1962 and 1963 described eight children with a wide range in degree of impaired mental development, all of whom had heparan sulfate mucopolysaccharidoses. Some of these patients had similarities, in appearance and in roentgenographic findings, to patients with Hurler and Hunter syndromes. The syndrome is characterized chemically by the exclusive excretion of heparan sulfate in the urine, which distinguishes it from all other mucopolysaccharidoses. It is also clinically unique in the disparity between the generally severe cerebral degeneration and the relatively mild effects on the skeleton, viscera, and facial features [4].

Fibroblasts derived from patients with the Sanfilippo disease accumulate $^{35}SO_4$. The existence of more than one type of disease was first recognized through cross-correction studies [5]. Patients studied initially fell into two groups, and those of each group could correct the other. The correction factors are the enzymes whose activity is lacking in each of the types. In type A Sanfilippo cells, Kresse and Neufeld [6] found that the defective enzyme is heparan-N-sulfatase (Figures 79.1 and 79.2). In type B, the defect was found by O'Brien [7] and by von Figura and Kresse [8] to be in a-N-acetylglucosaminidase (Figure 79.1). The latter group defined the acetyltransferase defect in type C [9] and the N-acetylglucosamine-6-sulfatase abnormality in type D disease [10]. The cDNA for this IIID disease gene has been cloned [11] and mapped to chromosome 12q14 [12]. The cDNA for the IIIA enzyme was cloned and mapped to chromosome 17q25 [13]. The gene for IIIB was cloned and mapped to chromosome 17q21 [14]. A relatively small number of mutations, predominantly missense and private to an individual family, has been found in the IIIA and IIIB genes [15, 16].

CLINICAL ABNORMALITIES

The clinical features of each of the four Sanfilippo disease types are indistinguishable (Figures 79.3–79.10). Patients are characteristically normal in appearance at birth and appear to develop normally during the first year. They are usually referred after one or two years of age because of slowness in development or after three or four years because of delayed speech. They may have had difficulty in feeding, especially with solids, as well as repeated respiratory infections from the beginning. Impaired mental development becomes progressively more obvious with time. These patients do not have abnormalities in linear growth, and muscle strength is good. Progressive degeneration leads to a severe degree of mental incapacitation, though there is variability among patients. Skills learned during the first years are lost, including speech and toilet training. Others never learn to speak,

while speech in some is lost well after the first decade; some patients develop some impairment of hearing. Neurologic problems are progressive. The gait becomes clumsy and coordination poor. Deep tendon reflexes are accentuated. Purposeless athetoid-like movements may develop. The patient may drool constantly. There may be seizures, but anticonvulsant control is not difficult. Finally, the patient becomes bedridden, and gastrostomy or nasogastric feeding is required. Death usually supervenes before the 20th birthday or even before the tenth [4], but survival into the third or fourth decade is possible. Median age at death was 18 years, usually of pneumonia [17].

Management of behavior may be a problem and may even be the presenting complaint. Behavior tends to become

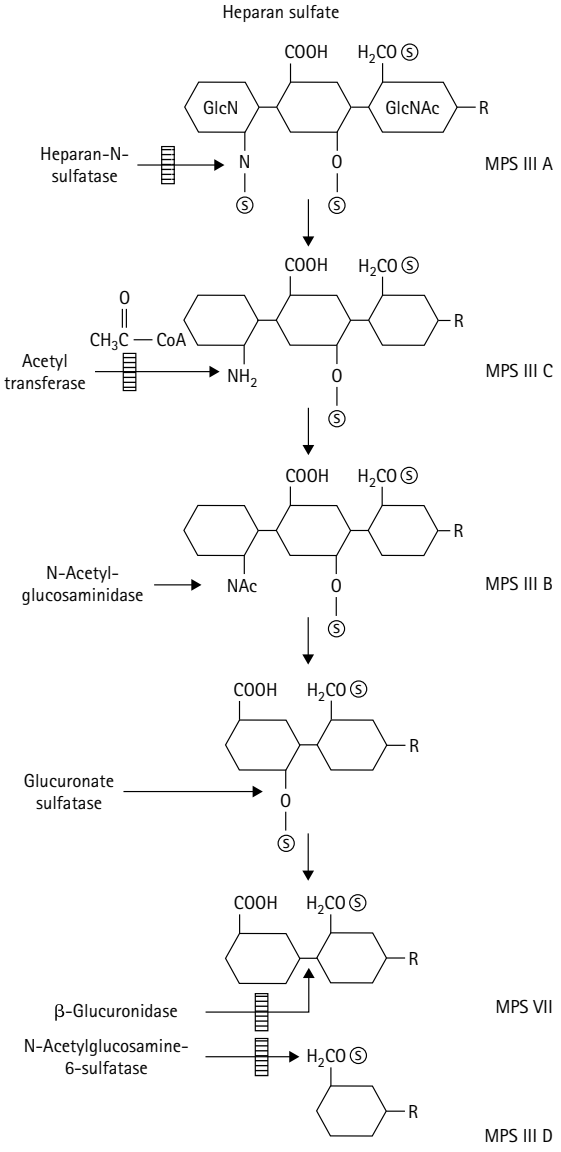

Figure 79.1 The defect in Sanfilippo disease type A is in heparan-N-sulfatase, while in type B it is α-N-acetylglucosaminidase. In type C, it is an acetyl transferase, and in type D, it is N-acetylglucosamine-6-sulfatase. The phenotypes of the various types are indistinguishable.

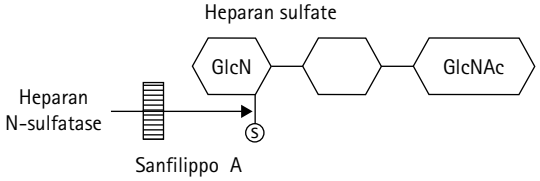

Figure 79.2 Heparan sulfate accumulates in Sanfilippo disease.

Figure 79.3 CF: An 11-year-old girl with Sanfilippo A disease. The thick alae nasi were clear evidence of mucopolysaccharide storage, but features were otherwise not coarse. She had moderate hirsutism and severe hypertonia, as indicated by her hands. Liver was palpable at 4 cm.

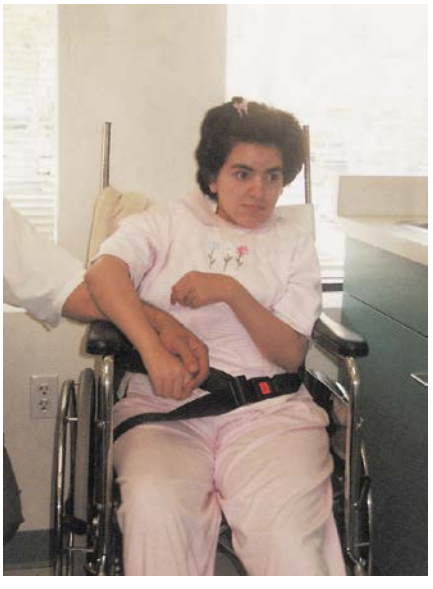

Figure 79.4 CL: A 21-year-old female with Sanfilippo A disease. She was thin, dystonic, and wheelchair bound.

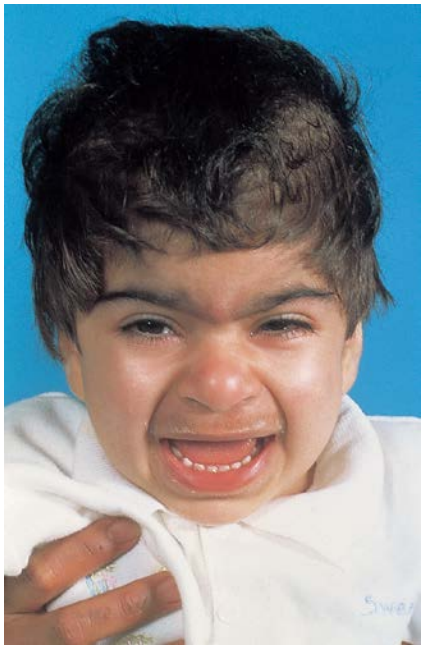

Figure 79.5 RGQ: A 21-month-old girl with Sanfilippo disease type A. She was quite hirsute, and the facial features were somewhat coarse. The liver was palpable at 8 cm. Growth was normal, but she was developmentally delayed.

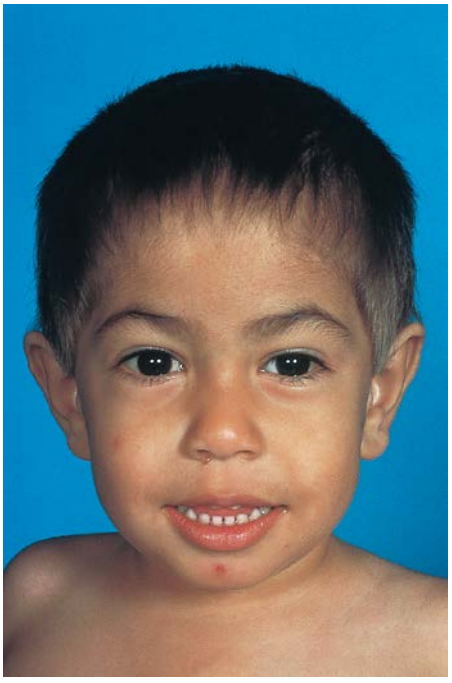

Figure 79.7 HMZ: A 2½-year-old boy with Sanfilippo disease type B. The diagnosis was made because of a positive family history. Features were not coarse, but he was more hirsute than the unaffected members of the family. Development was slow; he had only a few words, but he had walked at 18 months.

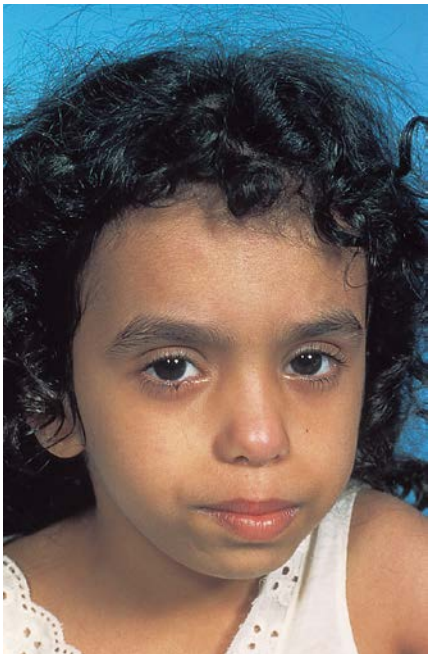

Figure 79.6 GGQ: A nine-year-old girl with Sanfilippo disease, the sister of the patient in Figure 79.5. The history was of loss of developmental milestones, such as walking. She was very hirsute. There was no organomegaly. The calvaria was thickened, but she did not have dystosis multiplex. On follow up by 16 years, she had profoundly impaired mental development and had spasticity; she had contractures and was unresponsive to social stimuli.

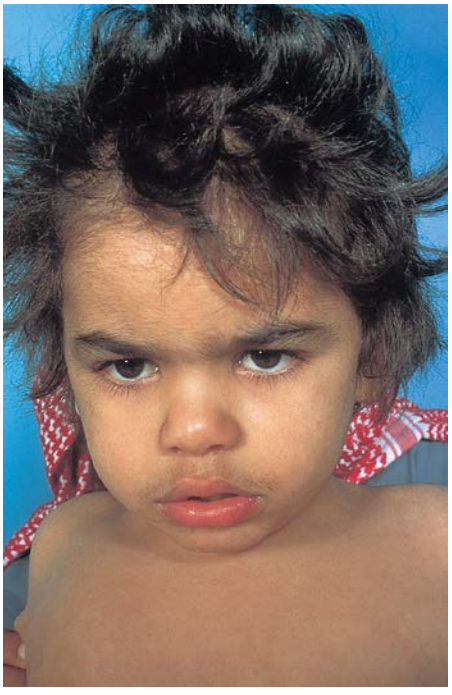

Figure 79.8 ARAZ: A four-year-old boy with Sanfilippo disease type B. Mental deficiency increased in severity after two years of age, and facial features coarsened. Hirsutism was prominent. By five years, his cognitive mental age on the Bayley scale was nine months, but motor performance was spared.

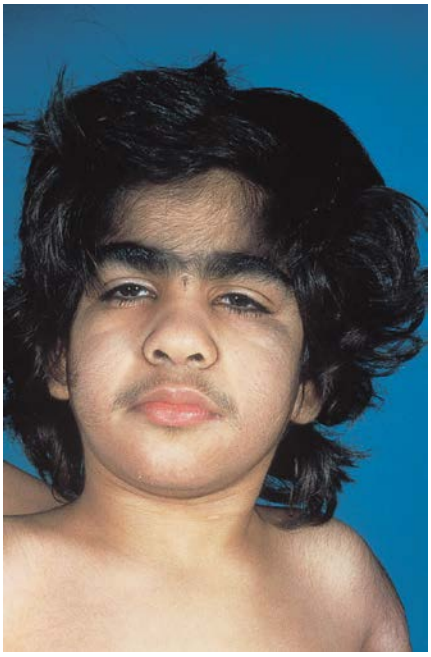

Figure 79.9 HRIH: A 12-year-old boy with Sanfilippo disease type B. He was hirsute, and his facial features were coarse, especially about the nose and lips; mental deficiency was severe.

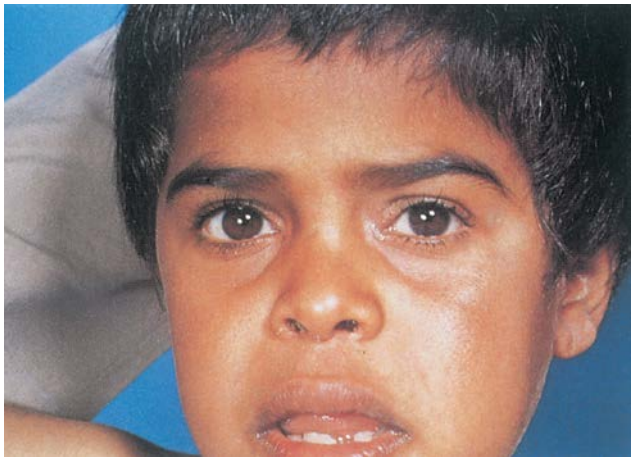

Figure 79.10 AS: A six-year-old girl with Sanfilippo disease type D. She had progressive intellectual deterioration. She had no speech, and she had autistic behavior, but facial features were unremarkable. Activity of N-acetylglucosamine-6-sulfatase was 5 percent of control.

worse as, with age, patients become increasingly stubborn and withdrawn; many are hyperactive. Disorders of sleep and insomnia are common, and some are up all night, at least on occasion. Chewing the bedclothes and sudden crying out are common. Inappropriate laughing or singing is less common. Patients may be aggressive, and temper tantrums occur. Patients may have pica and eat unusual objects. Interaction with other children may be difficult. They can be destructive and dangerous to siblings. The combination of aggressive behavior, profound dementia, and normal physical strength is a daunting one. They are often so difficult to handle that admission to an institution is common [18]. Some patients have come to attention as adults with psychiatric disease, even of a type requiring admission to a closed ward [19]. A neuro behavioral phenotype [20] has been observed in 99 percent of patients with Type A and B disease. In addition to autistic behavior, a Klüver-Bucy-type syndrome with reduced startle and loss of fear has been emphasized. Patients are at risk for harm to themselves and others [20]. Drug treatment of the behavior is seldom effective.

There may be differences in the severity of disease in the different types. Adult onset of dementia and minimal somatic disease have been reported in type B [21]; while dementia is commonly observed by six years of age in type A [18]. In addition, early-onset progression tends to be more rapid in type A than in types B and C. However, there is considerable heterogeneity. A particularly severe type A disease has been reported [22] from the Cayman Islands. In both types A and B, severe and mild forms of the disease have been reported in the same sibship [23, 24]. Type C severity may be intermediate between that of types A and B or may present in infancy [25]. Type D is rare, but also heterogenous [26–28].

The features of the patient with Sanfilippo disease usually become somewhat coarse (Figures 79.4–79.7), but often the patient is not recognizable as having a mucopolysaccharidosis. In some, the features are not recognizably coarse (Figures 79.9 and 79.10). In our experience, programs of screening of the urine for metabolic disease of unselected patients in institutions for the mentally impaired are more likely to bring to light previously undiagnosed patients with this disorder than any other disease of metabolism.

The bridge of the nose may be slightly flattened and the lips somewhat thick. Many of the patients are hirsute; the eyebrows may be bushy and the hairline low. Retinitis pigmentosa was observed in three patients with type C disease over 30 years of age [28].

Some have had macrocephaly. The dull, rigid facies is a consequence of cerebral deterioration, as contrasted with the local tissue changes of Hurler disease. Some patients may have a mild limitation of joint mobility. Hepatosplenomegaly may be mild, especially in childhood; it is more often undetectable in adulthood. There is no gibbus, and the corneas are clear. Cardiac abnormalities have not usually been observed in these patients [17]; however, a patient has been reported [29] in whom there was severe incapacitating involvement of the mitral valve. Hernias may be a problem and may recur after correction. Hearing loss may be progressive and severe. Watery diarrhea may be a recurrent problem in childhood. An early onset of puberty may be observed [30].

Roentgenographic findings are those of a mild dystosis multiplex (Figures 79.11–79.13) [17]. Most patients with this syndrome have a thickening and increased density of the cranial vault in the posterior parietal and occipital

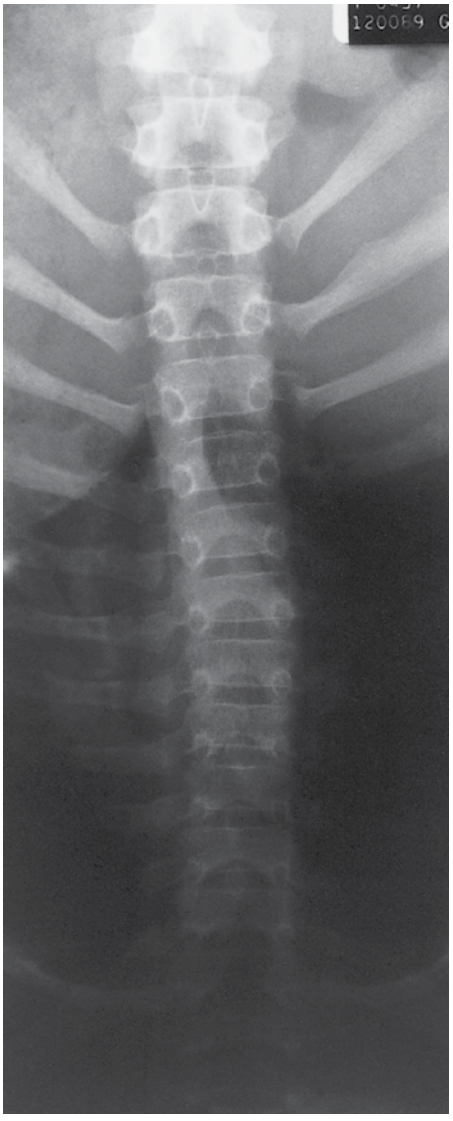

Figure 79.11 Roentgenogram of the spine of a 16-year-old boy with Sanfilippo disease. There was mild platyspondyly, and the ribs were spatulate with posterior narrowing. This is consistent with dysostosis multiplex, but appreciably milder than those of other forms of mucopolysaccharidosis. Roentogenograms of his extremities were normal.

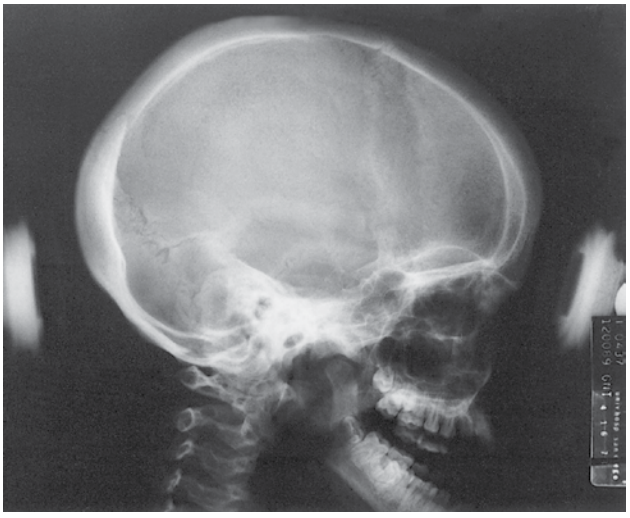

Figure 79.12 Roentgenogram of the skull of the 16-year-old shows increase in thickness of the diploic space, especially posteriorly. This is characteristic of this disease. The sella turcica is normal.

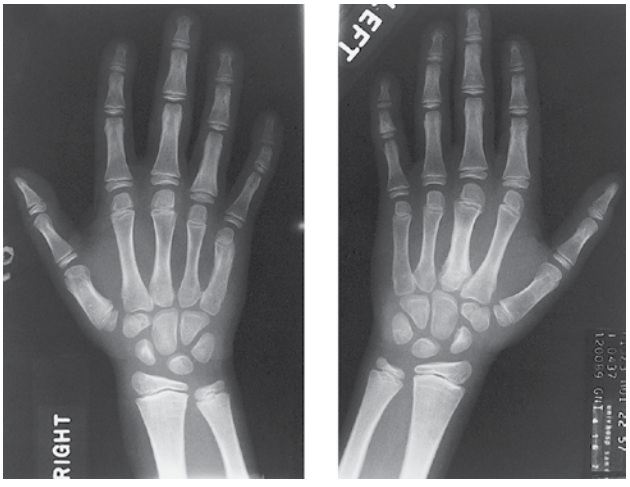

Figure 79.13 Roentgenogram of the hands of the same 16-year-old looks normal. This highlights the difference in the bones of this disease, from those of disorders with more severe dysostosis multiplex.

areas (Figure 79.12) [31]. The mastoids may be sclerotic. The sella turcica appears normal. There may be a biconvex appearance or an ovoid dysplasia of the thoracolumbar vertebrae, as well as platyspondyly (Figure 79.11). Among patients with dysostosis multiplex, those with this syndrome have the mildest bony changes. Those with I cell disease, or mucolipidosis II and Gm1 gangliosidosis, and Hurler disease have the most prominent bony changes. Computed tomography (CT) scans or magnetic resonance imaging (MRI) may reveal mild atrophy early, but this is progressive with the neurodegeneration.

Blood smears may reveal the presence of metachromatic inclusion bodies in the lymphocytes. They are characteristically coarser and sparser than those seen in Hurler disease. Inclusions may also be seen in cells of the bone marrow. Chondrocytes in cartilage biopsied from the iliac crest and the ribs have been reported to be vacuolated [32]. The diagnosis of a mucopolysaccharidosis is first made by the findings of increased quantities of mucopolysaccharide in the urine. In this disorder, it is heparan sulfate that is excreted in excess [33].

Patients with this disorder accumulate gangliosides in the brain [34, 35] including Gm2 and Gm3 [34], or there may increased amounts of Gm1 [35]. The electron microscopic appearance of the neurons may be like those of Tay-Sachs disease [34] (Chapter 88). There may also be zebra bodies, and mucopolysaccharide may accumulate in the brain, as well as in the peripheral tissues.

GENETICS AND PATHOGENESIS

All four types of Sanfilippo syndrome are transmitted in an autosomal recessive fashion. Multiple affected siblings have been observed in several families, and consanguinity has been documented [18]. The frequency of the disease has been estimated at one in 24,000 in the Netherlands [18, 36]. In this population, and in Great Britain [37], the most frequent type was A. In the Cayman Islands there is a very high prevalence of Sanfilippo A disease [22]; the carrier frequency is 0.1. In Greece, 10 of 11 patients reported were of type B [38]. An incidence rate for all forms in Australia was 1 in 58,000 live births [39].

The heparan sulfate molecule (Figure 79.1) consists of a series of glucuronic acid and iduronic acid molecules alternating with glucosamine residues [40]. The amino nitrogen of the glucosamine moiety may be either sulfated or acetylated, and the 6-hydroxyl may be sulfated. The stepwise degradation of heparan sulfate provides the sites for the defects in the various forms of Sanfilippo disease. Heparan-N-sulfatase, the site of the defect in Sanfilippo disease type A [6, 41], catalyzes the breakdown of the molecule by splitting off the sulfate groups linked to the amino group of glucosamine. The enzyme has been isolated and purified [42] and is formally a sulfamate sulfohydrolase. α-N-acetylglucosaminidase, the defective enzyme in type B, catalyzes heparan sulfate breakdown at the glucosamine to hexuronic acid linkage removing the N-acetylglucosamine generated by acetyl transfer in the IIIC reaction. This enzyme has been purified from human liver and urine, and the biosynthesis of the mature lysosomal enzyme has been elucidated [43–45]. In the sequential catabolism of heparan sulfate, removal of the sulfate in the reaction catalyzed by heparan-N-sulfatase exposes a terminal glucosamine moiety. This cannot be cleaved until it is acetylated, after which the reaction deficient in type B Sanfilippo disease comes into play. The acetylation is catalyzed by a specific N-acetyltransferase, and it is this reaction that is defective in type C Sanfilippo disease [9, 46, 47]. This is a two-step reaction in which the enzyme is first acetylated on the cytoplasmic side of the membrane and then transfers this acetyl group now inside the lysosome to a glucosamine. AcetylCoA does not cross the lysosomal membrane. Some patients with type C disease are defective in the second step and others in both steps [48–50].

The defective enzyme in type D Sanfilippo disease is in α-N-acetylglucosamine-6-sulfatase [50, 51]. Glucosamine residues in heparan sulfate can be either N-sulfated or O-sulfated, and there are specific sulfatases for each residue. Defective activity of either leads to accumulation and to the Sanfilippo syndrome. The glucosamine-6-sulfatase has been purified [52] and its cDNA has been cloned [11]. Its structure is homologous to other sulfatases. Immunoprecipitation studies have demonstrated cross-reactive material in the Sanfilippo B-syndrome [53]. The enzyme is also involved in the degradation of keratan sulfate, but patients do not excrete keratan sulfate, because this block may be obviated by other enzymes. Patients excrete N-acetylglucosamine sulfate, as well as heparan sulfate [54].

Within each of the forms, there is not only interfamilial variability, but also intrafamilial variability [55, 56]. Patients with all forms excrete heparan sulfate and no other glucosaminoglycan.

Detection of heterozygotes has been accomplished through enzyme assay [57] and by ^{35}S accumulation, which may be more reliable in distinguishing heterozygotes. Positive identification by enzyme assay is accurate, but there may be overlap, making identification of the normal noncarrier individual unreliable. When the mutation is known, molecular methods may be used for the detection of carriers.

Intrauterine diagnosis of the affected fetus has been accomplished in types A and B disease through assay of the relevant enzyme in amniotic cells in culture [58]. It may be useful to confirm the assay by assessment of ^{35}S accumulation, because some heterozygotes have very low levels of enzyme in the usual assays. Chorionic villus material has abundant enzyme activity and is available for the prenatal diagnosis of each of the forms of Sanfilippo disease [58–61].

The gene that encodes the heparan-N-sulfatase, defective in mucopolysaccharidosis type IIIA (MPS IIIA), has eight exons over 11 kb [13, 62]. Among the early mutations identified was an 11 bp deletion [13]. Some other deletions have been reported, but most mutations have been missense [15, 16, 63–66]. Among Australian, Dutch, and German patients, the most common mutation was R245H [17, 64]. Other common mutations are Q380R, S66W, and 1080delC, all with the severe phenotype. Among Polish patients, it was R74C [65], S66W in Sardinians [65], and 1091delC in Spaniards [66].

The gene for the acetylglucosaminidase defective in mucopolysaccharidosis type IIIB (MPS IIIB) contains six exons over 8.5 kb [14]. A number of mutations have been identified [14, 16, 67, 68]. A number of replacements of arginine by histidine or stop codon were found at CPG hotspots [14]. In six Japanese families, F314L and V241M were novel mutations [68]. Three patients were homozygous for R482W, R565P, R565W, and R565P.

The gene for N-acetyltransferase (HGSNA7) was identified and two mutations were found in human cell cultures [70]. A splice junction mutation accounted for three mutation alleles. Among Spanish patients the most common 50 percent mutation was 234+1G>A [71].

The gene for N-acetylglucosamine 6-sulfatase, on chromosome 12q14, the site of the defect in Sanfilippo type D, has been cloned and encodes a protein of 52 amino acids. The first mutation to be found in a consanguineous Pakistani family was a homozygous single base pair deletion c.1169delA [69]. A1bp insertion was reported [72] in a Turkish boy.

TREATMENT

There is no effective treatment for Sanfilippo patients. Enzyme replacement therapy has been attempted using

partially purified enzyme, leukocytes, and cultured fibroblasts [73], after which there may have been some changes in urinary mucopolysaccharides, but clinical benefit has not been evident except in soft tissues [74]. Bone marrow transplantation has been explored in Sanfilippo disease, as well as in other mucopolysaccharidoses, but results have not been impressive [75] in the Sanfilippo group, and the procedure is not recommended. The overwhelming experience with mucopolysaccharidosis type III (MPS III) is that transplantation of marrow or stem cells does not reverse the inexorable neurodegeneration of this disease. Loperamide hydrochloride may be useful in the management of diarrhea. The dose is 1–2 mg up to four times a day.

Outcome measures have been studied in a search for useful monitors for evaluating clinical trials [76]. The Mullen and Leiter scales of cognitive development and the parental report of adaptive behavior were judged best.

Preclinical assessment in mice of AAV based gene therapy by intracerebral injection has been explored [77, 78].

Behavioral modification may be useful in the management of problems of behavior. Otherwise, therapy is supportive.

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80

Morquio syndrome/mucopolysaccharidosis type IV/ keratan sulfaturia

MAJOR PHENOTYPIC EXPRESSION

Shortness of stature, pectus carinatum, dorsolumbar kyphosis, odontoid hypoplasia, genu valgum, corneal clouding, dental anomalies, aortic valve disease, and keratan sulfaturia. Activity of galactosamine-6-sulfate sulfatase is deficient in type A Morquio syndrome; lysosomal β-galactosidase is defective in type IV B.

INTRODUCTION

The syndrome was described by Morquio in 1929 [1] in four affected siblings in Uruguay who were the products of a marriage of first cousins of Swedish origin. In the same year, Brailsford [2] described a similar patient in England. The excretion of keratan sulfate in the urine is the defining biochemical feature of patients with this disease, and keratan sulfate has been documented to accumulate in tissues [3–5]. Defective degradation of keratan sulfate leads to its accumulation in those tissues in which it is normally abundant: cartilage, nucleus pulposus and cornea – tissues that are prominent in the clinical manifestation of the disease. Keratan sulfate consists of alternating galactose and N-acetylglucosamine residues; each may be sulfated. The molecular defect in type A or classic Morquio syndrome is in N-acetylgalactosamine-6-sulfatase encoded by the *GALNS* gene. It is also a galactose-6-sulfatase responsible for the cleavage of the galactose-6-sulfate moieties of keratan sulfate (Figure 80.1) [6–8]. This enzyme also catalyzes the removal of sulfate moieties from N-acetylgalactosamine-6-sulfate residues that are present in chondroitin-6-sulfate (C6S), and this leads to excretion in excess of chondroitin sulfate in Morquio syndrome. The accumulation of the KS and C6S, in bone and cornea leads to a systemic skeletal chondrodysplasia [9].

In the sequential degradation of keratan sulfate, once the first sulfate has been cleaved, the terminal galactose is cleaved in a reaction catalyzed by β-galactosidase encoded by the *GLB1* gene. This is the enzyme that is defective in type B Morquio syndrome [10–12].

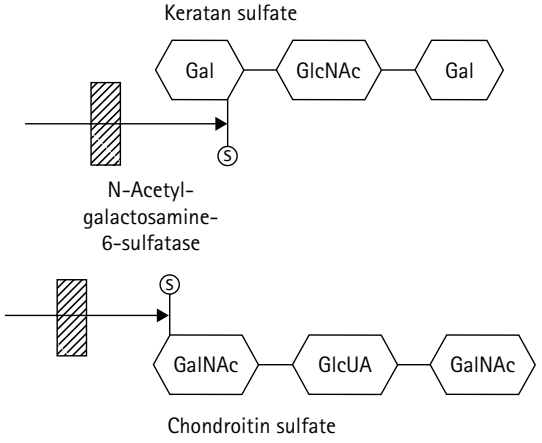

Figure 80.1 N-Acetylgalactosamine-6-sulfatase and the degradation of keratan sulfate. This is the site of the defect in type A Morquio syndrome. The enzyme also hydrolyzes the sulfate from the N-acetylgalactosamine-6-sulfate moieties that occur in chondroitin-6-sulfate.

The gene for the galactose-6-sulfatase deficient in type A Morquio disease has been cloned [13] and mapped to chromosome 16q 24.3 [14, 15]. A number of mutations has been described [16–19]. The gene for the β-galactosidase defective in type B has been localized to chromosome 3 p21-cen [20]. Some mutations have been defined [21].

CLINICAL ABNORMALITIES

The clinical pictures in types A and B are indistinguishable. In both, there is considerable heterogeneity ranging from mild to severe, including even hydrops fetalis phenotypes. The most characteristic features of this syndrome are skeletal deformities and shortness of stature, which is particularly short-trunked, though the long bones are also involved. The neck is short and the head appears to sit directly on the barrel chest, which classically has a very pronounced pectus carinatum (Figures 80.2–80.8). The upper part of the sternum may be almost horizontal. There is also a pronounced genu valgum, and patients often have a semi-crouching stance. The joints are enlarged and prominent (Figures 80.9–80.14). On the other hand, as a result of ligamentous laxity, there is usually extreme hypermobility and hyperextension of the joints, particularly at the wrists, where there may be marked ulnar deviation (Figure 80.10). Joints may become stiff with age. Pes planus is also seen.

These skeletal changes are not obvious during the first year of life because intrauterine growth and early extrauterine development are normal. Prominence of the lower ribs may first bring the patient in for medical consultation at 12 to 18 months of age. Others first come to attention because of prominence of the sternum. Flat

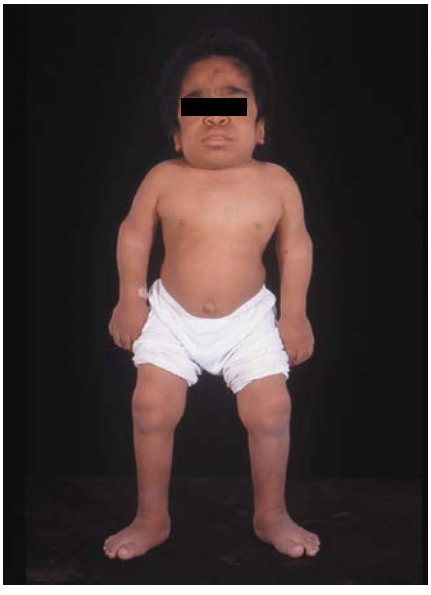

Figure 80.2 A 16-year-old patient with Morquio disease type A. He had normal intelligence. Illustrated are the typical pectus carnatum, deformed arms and legs, and stunted stature.

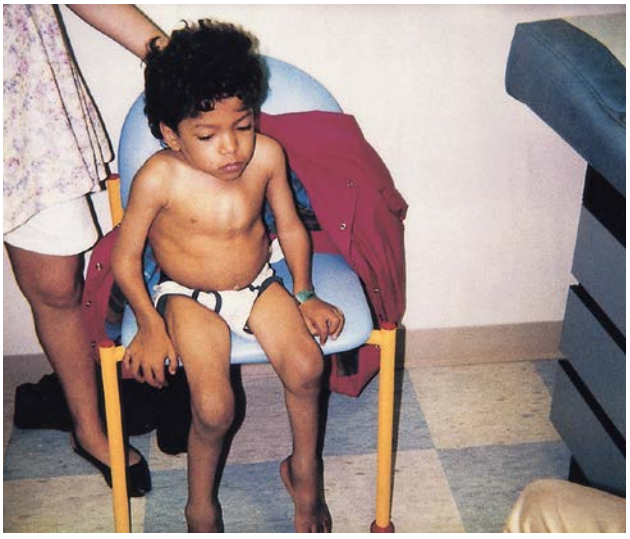

Figure 80.3 FD: A ten-year-old Honduran boy with Marquio syndrome. He was short and had a prominent pectus carnatum. The neck appeared very short.

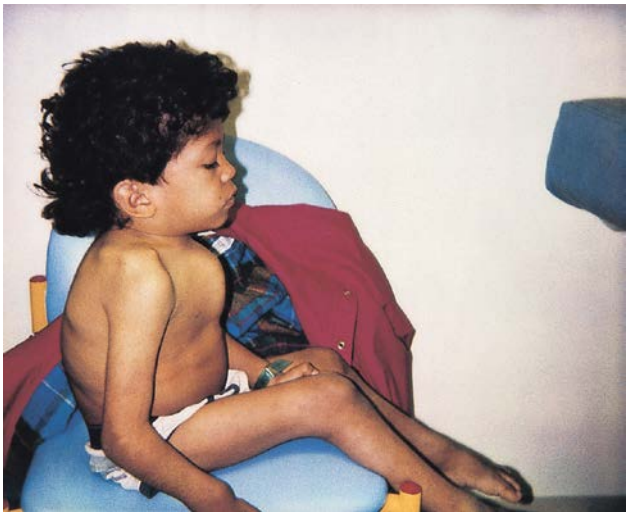

Figure 80.4 FD: In the lateral, the kyphosis and pectus are seen clearly. There was irregular flaring at the rib cage.

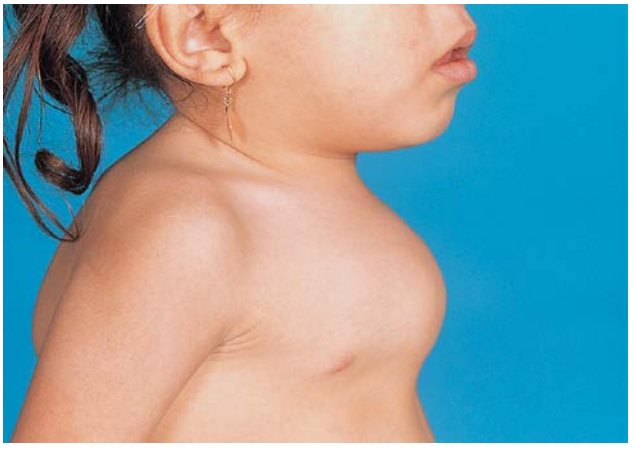

Figure 80.5 MKMT: A four-year-old with Morquio type II disease. The prominent pectus is shown.

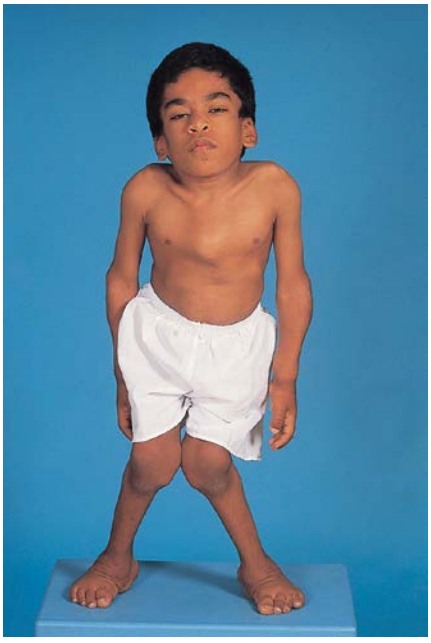

Figure 80.6 ABV: A 12-year-old boy with Morquio disease. He had kyphosis, as well as a prominent manubrium and a small thoracic cage. He had marked genu valgum and flexion deformities of the hip. Activity of N-acetylgalactosamine-6-sulfatase in fibroblasts was undetectable.

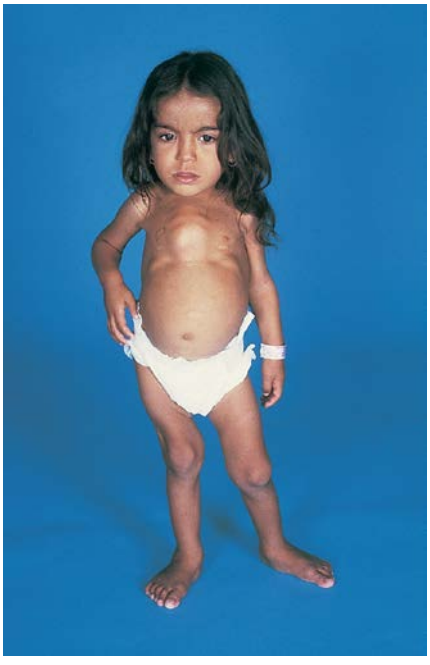

Figure 80.7 MAZ: A three-year-old girl with Morquio disease and the typical pectus deformity, short neck, and flat facies, as well as valgus deformities at the knees and ankles.

feet may be an early sign. In the second or third year, patients develop awkward gaits and impaired growth as skeletal deformities begin to be evident. The deformities are progressive and become exaggerated with age [22]. Patients with milder disease have presented in early adolescence

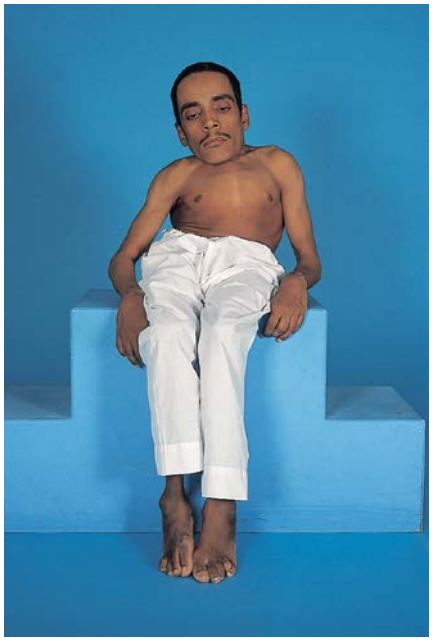

Figure 80.8 FRAM: A 26-year-old with Morquio disease and a spastic tetraplegia, a consequence of cord compression at C1 and C2 and odontoid hypoplasia.

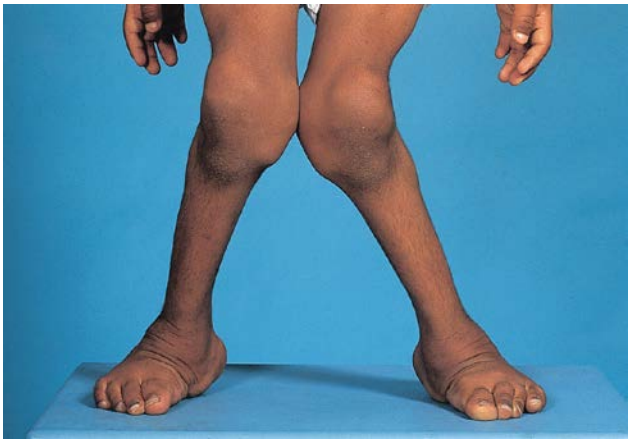

Figure 80.9 ABU: The genu valgum. The ankles and feet were quite broad.

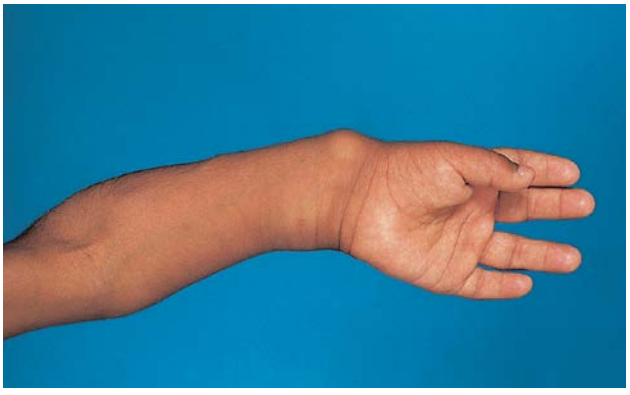

Figure 80.10 ABU: The wrists were very floppy and he had poor grip strength.

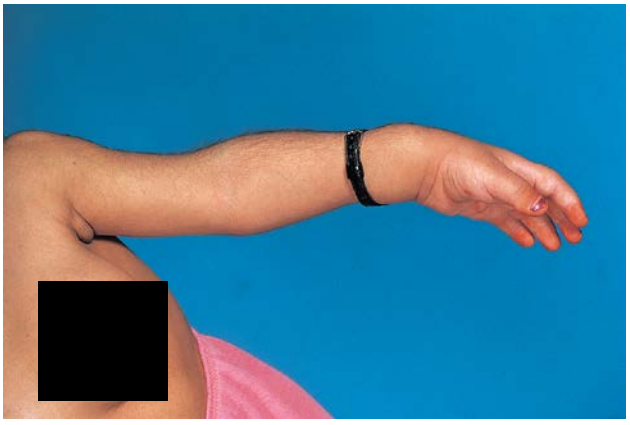

Figure 80.11 ABAO: The wrists of this 14-year-old patient were very floppy, and ulnar deviation was the result of the very short ulna.

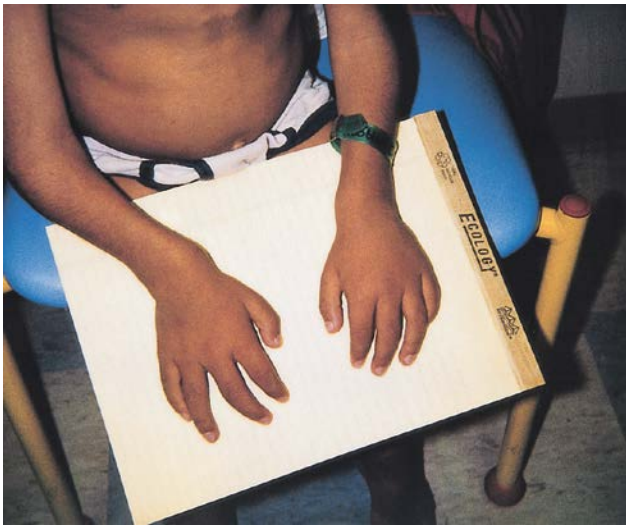

Figure 80.12 FD: The joints of the wrist and hand were enlarged.

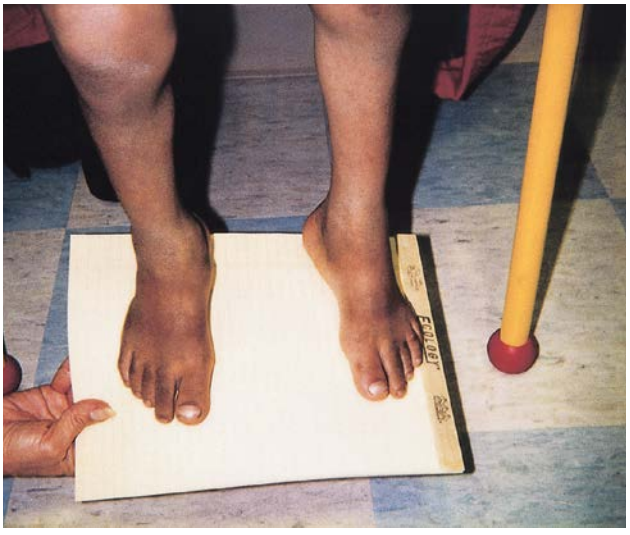

Figure 80.13 FD: The ankles and feet were also broadened.

with bilateral Legg-Perthes disease [23]. In similar fashion, a 30-year-old was reported with severe hip disease [24]. Growth is markedly slowed after about five years of age. Maximal height of 85–100 cm is usually reached by seven to eight years of age. Diagnostic delay is the rule and the diagnosis may not be made until as late as 15 years [25, 26].

These patients have fine corneal opacities, which are usually visible only on slit lamp examination, but may cause a hazy cloudiness of the cornea [3, 27]. Glaucoma was observed in siblings in their thirties [28]. Progressive sensorineural or mixed deafness usually begins in the second decade and is present uniformly after 20 years [29].

The mouth tends to be broad, and there may be spacing between the teeth, which may be small and flared [30, 31]. The enamel is hypoplastic both in deciduous and in permanent teeth. The teeth develop a gray or yellowish color, and the enamel becomes flaky or fractured. Molars are tapered and often have sharp cusps. The teeth easily develop caries. In one series [32], dental changes were observed only in type A, not in type B. A regular later manifestation of the disease is aortic regurgitation [33]. Severe scoliosis may lead to cardiorespiratory complications. Three of Morquio's original patients died of pulmonary complications. Inguinal hernia is probably more common than in normal individuals. The brain is normal and patients usually have normal intelligence. Ian Smith, a three feet tall, 14-year-old with Morquio syndrome played the title role in the Disney film Simon Birch and speaks publicly in support of rare diseases. Facial features may be somewhat coarse, the chin prominent and the mouth wide. The neck is short (Figures 80.2 and 80.3). Roentgenographic findings (Figures 80.15–80.17) in Morquio disease vary with the age of the patient [34]. The most characteristic and consistent finding is the universal platyspondyly or vertebra plana, which produces the short spine The vertebral bodies are usually oval-shaped in the younger affected child, becoming flatter and more rectangular in later childhood and flat in the adult. The cervical spine is striking in that the odontoid process of C2 is either absent or hypoplastic [35]. The remainder of the cervical vertebrae are flat. The thoracic and lumbar vertebrae show flattening and anterior beaking or tonguing. L1 is often short, anteriorly wedged, and displaced posteriorly, accounting for the gibbus. Patients with this syndrome always have a marked coxa valga deformity (Figure 80.15). The pelvis is narrow. With age, the anterior portions of the ribs become wide and spatula-shaped. The sternum protrudes. The femoral head becomes progressively flattened and fragmented; it may be completely resorbed. The femoral neck initially loses its angle and later becomes thickened. The distal femur is wide, as is the proximal tibia. These changes contribute to the production of the genu valgum that is characteristic of this disease. The distal end of the humerus is wide and irregular, as are the proximal ulna and radius – changes similar to the corresponding bones of the lower extremities. The growth plates of the distal ulnae and radii are slanted toward each other, the ulna usually being somewhat shorter

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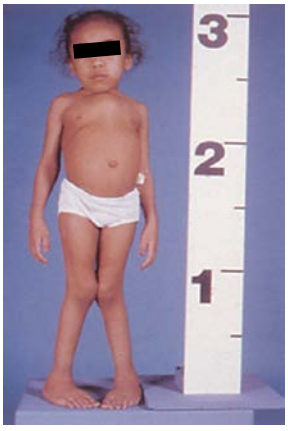

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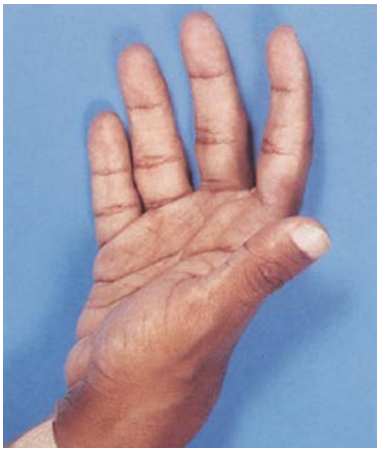

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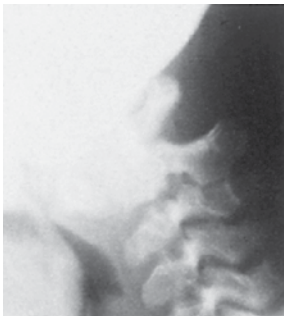

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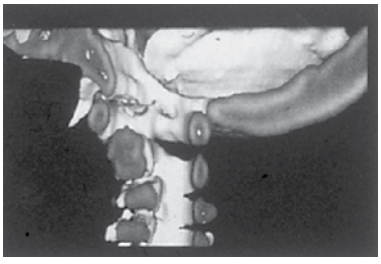

Figure 80.14 A seven-year-old girl with Morquio type A with typical phenotype (A). Her floppy wrist are shown (B). Roentgenograms show odontoid hypoplasia and C1–C2 posterior dislocation (C and D). A unique molecular defect was found. The family underwent preimplantation genetic diagnosis and had a normal baby [45].

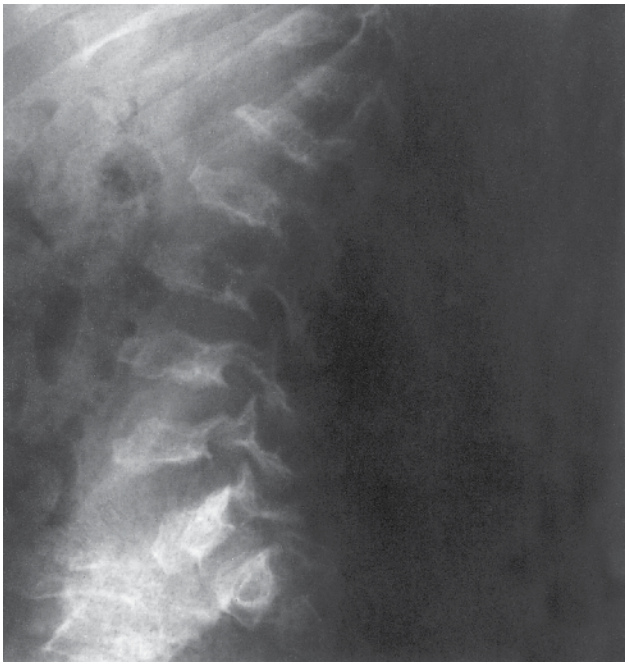

Figure 80.15 Roentgenogram of the spine of a five-year-old boy with Morquio syndrome. The vertebral bodies were very flat and beaked anteriorly. The second vertebra from the top was hypoplastic and displaced posteriorly. This is the genesis of the gibbus in this syndrome. (Illustration was kindly provided by Dr David Rimoin, University of California Los Angeles and Cedars of Lebanon Hospital, Los Angeles, California.)

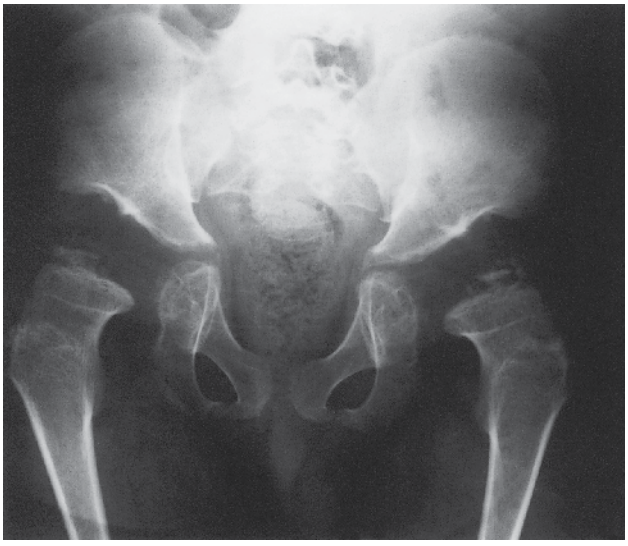

Figure 80.16 Roentgenogram of the pelvis of the same patient. The capital femoral epiphyses were flattened and irregular. There was coxa valga. The lateral margins of the acetabula were hypoplastic, creating, in essence, large acetabula extending to the anterior superior iliac spine. (Illustration was kindly provided by Dr David Rimoin.)

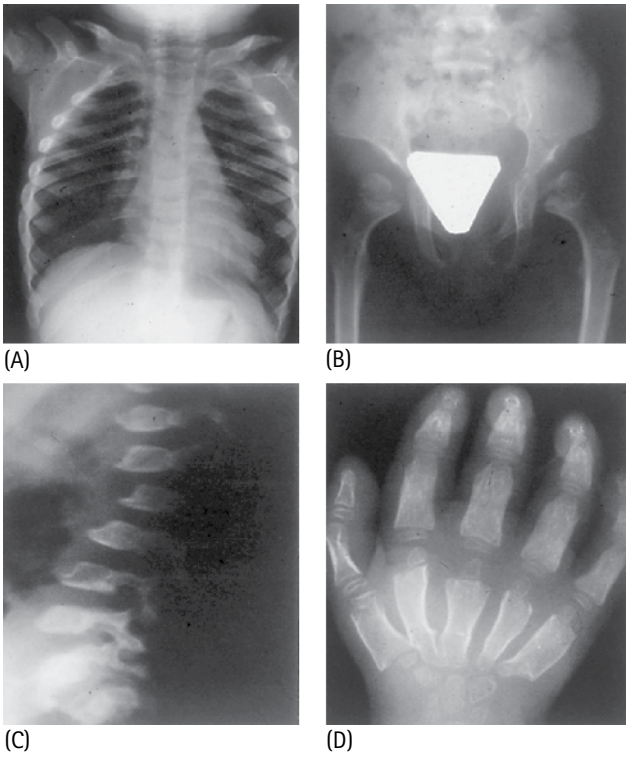

Figure 80.17 The bony abnormalities involved spatulate ribs (A), the typical pelvis with flattened femoral epiphyses and hypoplastic acetabular lateral margins and coxa valga (B). Posterior scalloping of the vertebrae and gibbus deformity are shown (C). Roentgenogram of the hand illustrated flattened carpal bones and proximal tapering of flexed metacarpals (D).

than the radius. The ossified carpal bones are small and may be reduced in number. The metacarpals are short and their distal metaphyses are widened. Osteoporosis is common in the adult patient.

Ocular manifestations of disease in 20 patients include corneal opacification (13/20), astigmatism (12/20), and cataract (6/20) [36]. Visual acuity improved with ophthalmologic correction.

A very dangerous complication of the bony deformity in this syndrome is that the spinal cord may be compressed following atlantoaxial subluxation or dislocation [12, 37, 39]. This is a major cause of death. Manipulation of the head for intubation may be particularly risky in these patients for this reason and anesthesia should be planned with this problem in the forefront, but subluxation may occur even during sleep and lead to death. This propensity for subluxation is attributed to the hypoplasia of the odontoid process and to the general laxity of the ligaments. These features are present in all patients with the disease, who therefore sooner or later may all expect to experience a complication of compression of the spinal cord. Neurologic manifestations may include weakness and difficulty in walking, uselessness of the legs on awakening, or spastic paraplegia (Figure 80.8). Loss of vibratory sensation in the lower extremities may be an early sign, and many patients have hyperactive deep tendon reflexes. Spinal cord compression may also occur at the level of the thoracolumbar gibbus. Subluxation of vertebra C2 to C3, as well as C1 to C2, has been observed [40] in type B patients.

The roentgenographic appearance may suggest avascular necrosis of the femoral head or spondyloepiphyseal dysplasia [41]. Mineral density of bone is uniformly abnormal; evaluation of the lateral distal femur is the most reliable [42].

Conjunctival biopsy may show intracytoplasmic vacuoles indicating lysosomal storage [43]. Metachromatic granules may be seen in the polymorphonuclear leukocytes or cultured fibroblasts [44]. Storage vacuoles have been demonstrated in the skin and in chondrocytes [45]. There is minimal evidence of storage in the brain. Generally, mucopolysaccharidosis (MPS) IVA patients with a severe form do not survive beyond the second or third decade of life, whereas those patients with an attenuated form may survive over many years [9].

Growth curves have been developed [46] from 354 patients with Morquio A disease. Mean height at 18 years was 122 cm for males and 113 for females; both -7SD. Despite major neurologic impairment, cognition is usually normal [47].

GENETICS AND PATHOGENESIS

Morquio syndrome is transmitted by autosomal recessive genes. It has been reported on several occasions that normal couples have produced multiple involved siblings. Parental consanguinity has been documented [22]. Prenatal diagnosis may be performed by the assay of either enzyme in cultured amniocytes or chorionic villus tissue [48]. An effective preventive strategy for Morquio disease and other lysosomal storage disorders is preimplantation genetic diagnosis which has been carried out on 3-day embryos in Saudi Arabia with an almost 90 percent success rate [49].

Patients with Morquio disease characteristically have increased concentrations of keratan sulfate in the urine [50]. Levels of the acid mucopolysaccharide which does not contain uronic acid are often two to three times the normal amount. With age, the concentrations of this compound in urine decrease. An immunoassay for keratan sulfate is capable of diagnostic assay of either blood or urine in any age group and permits distinction of mild and severe phenotypes [51]. More recently [52], a liquid chromatography tandem mass spectrum method has been developed for the assessment of keratan sulfate levels in blood. Levels varied with age, as well as severity of disease. The method is useful for diagnosis and the evaluation of severity of disease. Absence of keratan sulfaturia has been documented in enzyme-proven type B [11] and type A [23, 24, 32] Morquio disease.

In Morquio type A, the defective enzyme catalyzes the removal of 6-sulfate moieties from galactose, and from the N-acetylgalactosamine residues of chondroitin

sulfate. This latter property gave the enzyme its name. N-acetylgalactosamine-6-sulfatase has been purified from human placenta, and the defective enzyme has been demonstrated in cultured fibroblasts and in brain [3, 5, 8]. Deficiency of the type A enzyme has been demonstrated in patients not excreting keratan sulfate [53]. In five patients studied immunochemically, no cross-reacting material was demonstrated [54], but cross-reactive material has been demonstrated immunochemically in both Morquio type B and type A [55]. The full length of cDNA has been cloned and sequenced for human N-acetylgalactosamine-6-sulfatase [13], and transfection into deficient fibroblasts led to activity. The type A gene has 14 exons, and the sequence of 522 amino acids of the enzyme has considerable homology with other sulfatases, such as iduronate-2-sulfatase. At least 16 polymorphisms have been identified in the *GALNS* gene [56]. Polymorphic haplotypes may be employed for carrier detection and prenatal diagnosis in informative families [56], and this may be useful when mutations have not been identified. A considerable number and variety of mutations have been found in the Morquio type A gene [57].

Most have been missense point mutations, but there were a few nonsense and splice site mutations and small deletions. Insertions and large rearrangements were rare. However large deletions as well as splicing defects were identified by novel methods [58].

Severe disease was present in patients with a T to C change at nucleotide 468 resulting in V138 A and a C to T transition at 386 substituting a cysteine for arginine 386 [18]. A two-base deletion at 1342delCA was also associated with severe disease [59]. I113F was found to be a common missense mutation in Caucasian, particularly in Irish patients [16, 57], as was T312S. Another novel mutation c.1567T>G was found in a Chinese patient located in the termination codon TAG. This change of TAG to GAG extended the peptide chain by 92 amino acids; marked change in the protein structure led to an impressive reduction in enzyme activity [60]. In 24 unrelated Chinese patients with Morquio type A disease, 27 mutations were found [59], of which 16 were novel; there were two splicing mutations (c.567-1G>T and c.634-1G>A), two nonsense mutations (p.W325X and p.Q422X), and 12 missense mutations (p.T881, p.H142R, p.P163H, p.G168L, p.H236D, p.N289S, p.T312A, p.G316V, p.A324E, p.L366P, p.Q422K, and p.F452L). The p.G340D mutation was common, accounting for 16.7 percent of Chinese mutant alleles [61].

By 2005 148 different mutations had been described [62] in type A. They were distributed along the entire gene. The ten most frequent were represented by single nucleotide changes and accounted for 35% of all mutations. Some 101 mutations were associated with a severe phenotype.

Some mutations in the β-galactosidase gene have been identified in genetic compounds [63, 64, 65]. Depending on the mutation, the phenotype can vary from that of severe GM1 gangliosidosis (Chapter 89) to Morquio disease type B [21]. In a patient with Morquio B disease, p.Y333H was found on one allele [65]; p.R201H was on the other. The latter allele has been associated with poorly transported protein products through the endoplasmic reticulum.

The diagnosis is best made by assay of cultured fibroblasts or leukocytes using a substrate derived from chondroitin-6-sulfate for the sulfatase [66, 67] and using p-nitrophenyl- or 4-methylumbelliferyl-β-galactoside for the β-galactosidase. Enzyme activity may be assessed by tandem mass spectrometry of blood spots and used in programs of newborn screening [68]. Novel specific substrates have been developed for newborn screening for Morquio A disease, and also for Maroteaux-Lamy disease [69].

In addition to types A and B, there are other clinical examples of Morquio syndrome, usually mild, in which defects in neither of these enzymes can be detected. These are non-keratan sulfate-excreting patients [70]. The skeletal deformities and other symptoms in these patients are similar to those seen in Morquio syndrome, but less severe. There is platyspondyly, genu valgum, flat feet, pectus carinatum, and flat, fragmented femoral heads. The pathogenesis of disease is not explained simply by the storage of material in chondrocytes. It has been proposed that accumulation in macrophages within cartilage canals and inadequate regression of canals could contribute to cartilaginous disease [71].

TREATMENT

Surgical fusion of the cervical spine may be life-saving in the prevention of spinal cord compression [72]. Surgical decompression may be required for cord compression. There is a tendency for the prognosis to be better in females. Osteotomies may be useful in correction of the genu valgum [70, 73]. Any surgery should be undertaken with caution because of the risk of atlantoaxial instability and because of the deformity of the chest and its effect on cardiopulmonary function [74]. Hearing aids may be useful [29]. The instability of the wrists, which makes working with the hands very difficult, may be improved by the use of wrist splints.

Enzyme replacement therapy is under exploration in knockout mouse models. In a study of recombinant human (rh)GALNS in MPS IVA fibroblasts, there was dose-dependent uptake via mannose-6-phosphate receptor mechanism and restoration of enzyme activity. In MPS IVA chondrocytes, rhGALANS was taken up and internalized into lysosomes. Enzyme activity previously undetectable became normal and there was decreased storage of keratan sulfate. In wild-type mice, intravenous injection of rhGALNS yielded distribution throughout cardiac valves and the growth plate [75].

Elosulfase alfa intravenous treatment of patients with advanced disease showed excellent reduction of urinary keratan sulfate [76]; 8 patients were clinically improved. Intrathecal heparan-N-sulfatase led to decline in CSF heparan sulfate. Clinical effects were unremarkable.

Chaperone therapy has been explored in patient fibroblasts; lipid accumulation improved and enzyme protein degradation was inhibited [77]. Derivatives of 4-epi-isofagomine were found to inhibit human β-gangliosidosis and enhance activities of mutant β-gangliosidase in patient derived cell lines [78].

Gene transfer therapy has been studied by transduction of human MPS IVA fibroblasts using adenoassociated virus (AAV)-based vectors, which carry human *GALNS* cDNA [80]. Activity levels of *GALNS* of 15–54 percent of wild type were observed. In murine MPS IVA chondrocytes, enzyme activities following transduction were up to 70 percent of control. Cotransduction with the sulfatase-modifying factor 1 (SUMF1) gene led to a further four-fold increase in enzyme activity [81]. Improved targeting to bone was obtained in Morquio A mice by bioengineering human *GALNS* to extend the N-terminus. Mice treated from the neonatal period had appreciable reduction of storage in tissues [81].

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81

Maroteaux-Lamy disease/mucopolysaccharidosis VI/N-acetylgalactosamine-4-sulfatase deficiency

MAJOR PHENOTYPIC EXPRESSION

Shortness of stature, limitation of joint motion and contractures, corneal clouding, hepatosplenomegaly, dysostosis multiplex, excretion of dermatan sulfate, and deficiency of N-acetylgalactosamine-4-sulfatase (arylsulfatase B).

INTRODUCTION

A distinct mucopolysaccharidosis was first recognized by Maroteaux, Lamy, and colleagues [1] in 1963 as a syndrome in which patients displayed some of the features of the Hurler syndrome, but had normal intelligence [1, 2]. Furthermore, the mucopolysaccharide found in the urine was predominantly dermatan sulfate. A number of variants have now been described in which the range of severity is quite broad.

The molecular defect (Figure 81.1) is in the enzyme N-acetylgalactosamine-4-sulfatase [3, 4]. It catalyzes the removal of sulfate moieties from both dermatan sulfate and chondroitin-4-sulfate. This protein is the Maroteaux–Lamy corrective factor [5]. The human cDNA has been cloned [6] and the gene has been mapped to chromosome 5q13-14 [7]. A variety of mutations have been identified [8, 9].

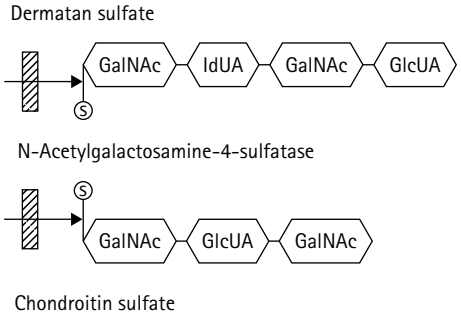

Figure 81.1 Degradation of dermatan sulfate and chondroitin sulfate. N-acetylgalactosamine-4-sulfatase, the site of the defect of Maroteaux-Lamy syndrome, is active in the degradation of both glucosaminoglycans.

CLINICAL ABNORMALITIES

The classic patient with the Maroteaux–Lamy syndrome develops impressively short stature [10, 11]. The patient is often first brought to the physician at two to three years of age because of impaired growth. The problem involves both the trunk and the extremities. By this time, the patient may be found to have the deformities and facial characteristics of a mucopolysaccharidosis (Figures 81.2–81.11). The facial features are recognizably coarse (Figures 81.2, 81.3, and 81.6), but they are considerably more subtle than those of the patient with Hurler syndrome. The breathing may be noisy from early infancy. A large head or prominent chest may be present at birth. There may be umbilical or inguinal hernias, and surgical repair may be required in the first years of life [10].

Ultimate height of 107–138 cm may be reached by six to eight years, the age at which growth usually stops. The head appears larger than the body. The skin may appear tight. Hirsutism is common. There may be macroglossia and protrusion of the tongue. The typical appearance of

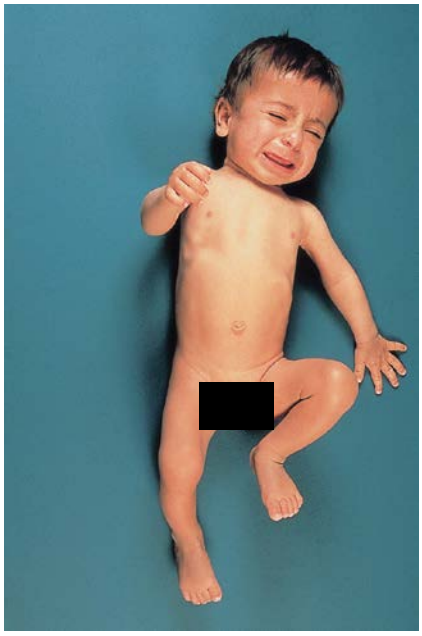

Figure 81.2 TSMG: A one-year-old Saudi Arabian boy with the Maroteaux-Lamy syndrome. He was very short. The facial features were coarse, but much less so than in Hurler syndrome or in most patients with Hunter syndrome.

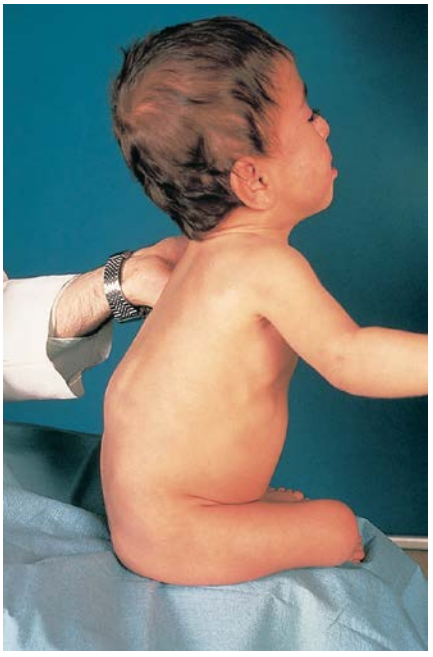

Figure 81.4 TSMG: In the lateral view, the gibbus is characteristic.

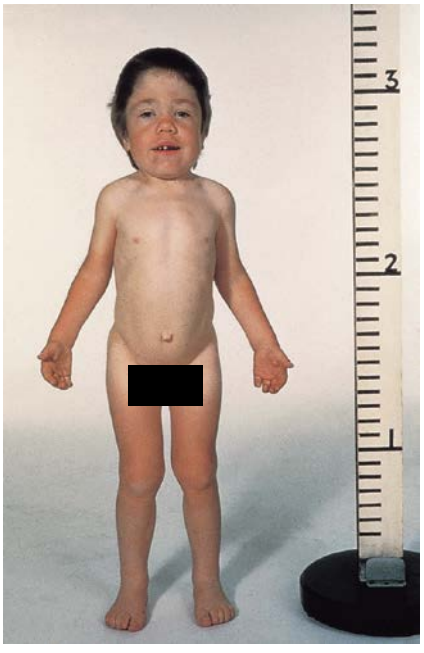

Figure 81.3 A seven-year-old boy with Maroteaux-Lamy disease. Illustrated are the shortness of stature, somewhat coarse facies and genu valgum. He also had cloudy corneas. (Illustration was kindly provided by Dr Philip Benson.)

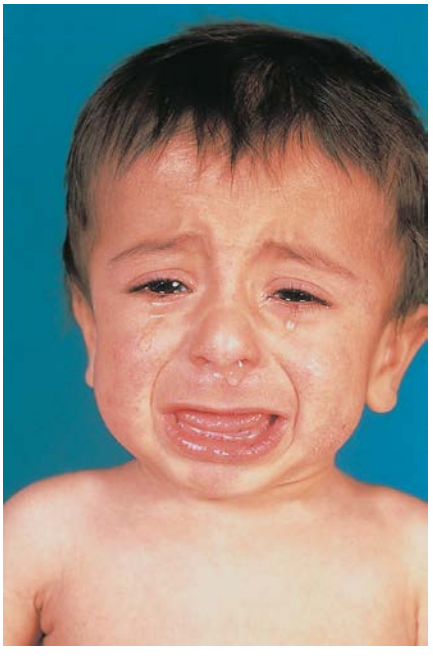

Figure 81.5 Close up of the face reveals coarse features, a flattened nasal bridge, and a large tongue.

the child with this disorder is of a short trunk, protuberant abdomen, and lumbar lordosis.

Changes of the joints are progressive, and motion becomes increasingly limited. Genu valgum and a position of semiflexion of the knees are characteristic, giving the child a crouched stance. A claw-hand deformity develops that is typical of mucopolysaccharidosis (Figure 81.9). There may be flexion contractures of the fingers, as well as the knees and elbows. The differential diagnosis may suggest mucolipidosis III (Chapter 83). A carpal tunnel syndrome may contribute to the limitation of hand motion. The subcutaneous tissues of the volar surfaces of the second

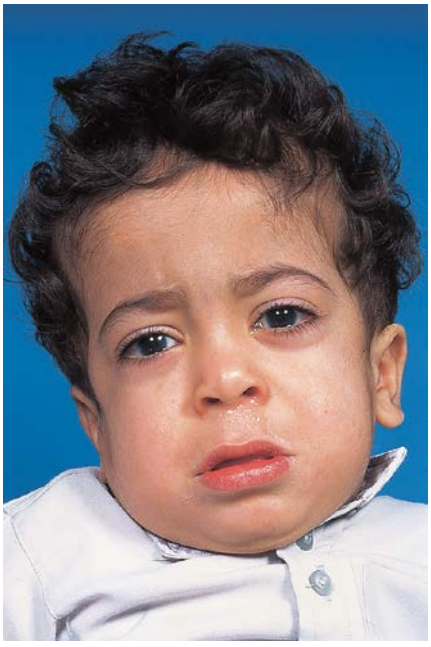

Figure 81.6 TSMG: At 18 months. The features were increasingly coarse.

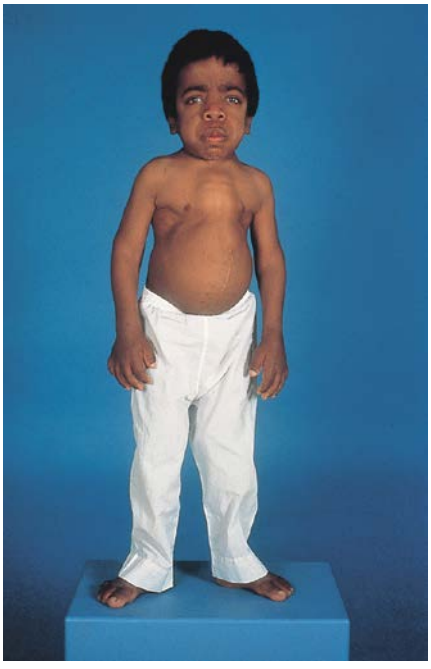

Figure 81.7 HAH: A nine-year-old Saudi boy with Maroteaux-Lamy disease. Facial features were coarse. He was very short. The activity of arylsulfatase B in fibroblasts was 4 percent of control.

to fourth fingers may be thickened, as in Dupuytren contractures [10]. Lumbar kyphosis and anterior protrusion of the sternum are also progressive.

Hepatomegaly is regularly observed in patients over six years of age, and splenomegaly is found in about half of the patients. Some patients have had frequent episodes of diarrhea.

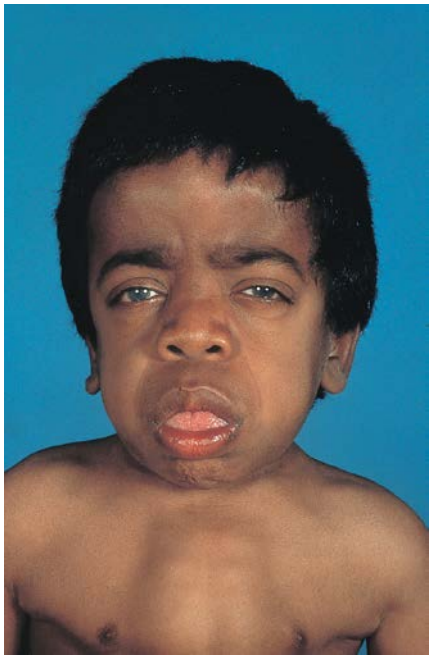

Figure 81.8 HAH: Close up of the face illustrates the low hair line, flat, coarse facies, and macroglossia. He had bilateral corneal clouding.

Figure 81.9 TSMG: At 18 months. The hand was typical of a mucopolysaccharidosis with broadening of the digits and claw-shaped contractures.

Cardiac abnormality is an important component of this syndrome [12]. Murmurs heard indicate valvular involvement. The mitral and aortic valves become thickened, calcified, and stenotic [13, 14]. A murmur of aortic stenosis is frequently present [15–17], and mitral or aortic regurgitation may also be present. Among 28 patients, 74 percent had abnormalities of the EKG and most had valvular abnormalities [18]. There may be right as well as left ventricular failure. An unusual presentation is with acute infantile cardiomyopathy [19].

Cardiac failure may be the cause of death, which usually occurs before 30 years of age in the classic form of the

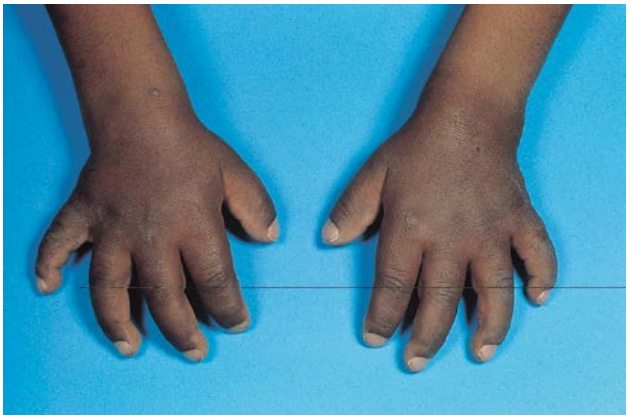

Figure 81.10 HAH: At nine years; the claw-hand deformity was very prominent.

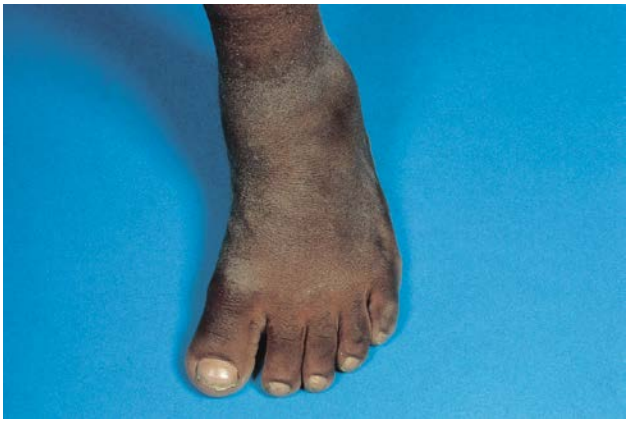

Figure 81.11 HAH: The foot was also broad and the toes particularly wide.

disease. Some have died of pulmonary infection. Another pulmonary complication is obstructive sleep apnea.

The corneas develop opacities at an early stage that are detectable by slit lamp examination and progressively become clinically cloudy. This is especially dense at the periphery, and it may lead to visual impairment. Glaucoma was reported in four adult women [20]. Deafness is a regular feature, related at least in part to recurrent otitis media.

In contrast to most of the mucopolysaccharidoses, Maroteaux–Lamy disease is characterized by normal intelligence. Two families have been reported in which there was impaired mental development, but this may have had some other etiology [21, 22].

On the other hand, neurologic complications occur frequently [23–25]. Hydrocephalus may result from pachymeningeal thickening. Ventricular shunting may be required. Myelopathy may result from cord compression following atlantoaxial subluxation. The dura may also be thickened in the cervical region, leading to insidious compression of the cord. The end result is spastic paraplegia [26]. Myelopathy due to compression may also result from developmental abnormalities of the vertebral bodies and kyphoscoliosis [27]. Spastic quadriparesis was found to result from hypertrophy of the ligamentum flavum causing widespread cord compression [28]. Papilledema and progressive loss of vision may be a consequence of increased intracranial pressure [11]. Neurologic deterioration has been observed during pregnancy [24]. A complete lack of development of secondary sexual characteristics, as well as an unusual degree of dwarfism in a patient, suggested that the anterior pituitary was also affected.

Patients with milder variants may present with hip dysplasia resembling Legg-Perthes disease. Some present first as adults with disease of the hips [29].

Roentgenograms demonstrate the typical findings of dysostosis multiplex (Figures 81.12–81.14) [11]. Roentgenograms of the hand may in classic examples be indistinguishable from that of Hurler disease (Chapter 76).

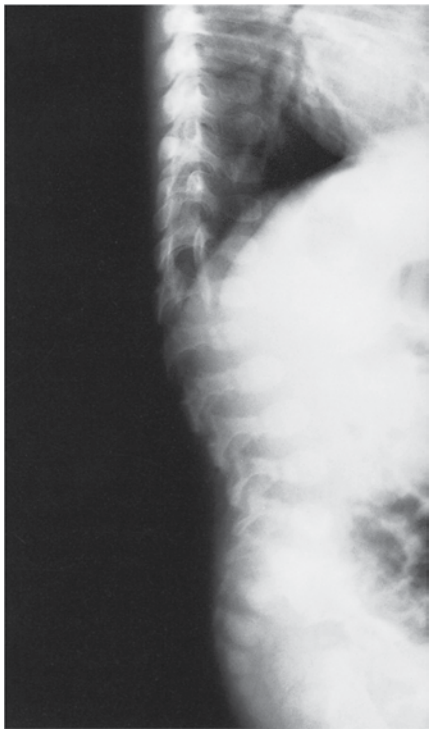

Figure 81.12 TSMG: Lateral roentgenogram of the spine. The vertebral body of L1 was hypoplastic and prominent anteriorly. The gibbus deformity was in this area.

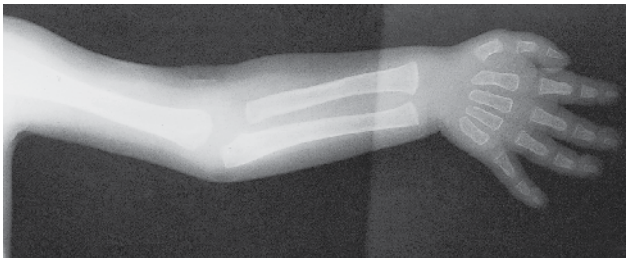

Figure 81.13 TSMG: Roentgenogram of the arm. The bones are all thickened and poorly modeled.

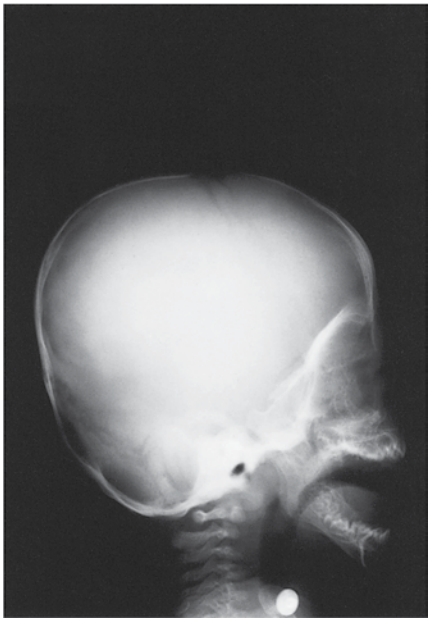

Figure 81.14 TSMG: Roentgenogram of the skull. The cranial vault was high and the sella J-shaped. Adenoid tissue was prominent.

In some patients, diaphyseal constriction may suggest Morquio disease (Chapter 80). The epiphyses are abnormal. Femoral heads may be particularly dysplastic, and coxa valga is a regular occurrence. The iliac bodies tend to be small and constricted, and the wings flare. The acetabula are small and hypoplastic and the acetabular roofs are oblique. Ossification of the femoral head may be irregular, and this is the reason patients have been thought to have Legg-Perthes disease. The femoral necks may turn outward. Widening of the epiphyseal plates may resemble metaphyseal chondrodysplasia.

The long bones are short and thickened or distended (Figures 81.13) and the radius and ulna may be bowed. In addition, there may be a localized metaphyseal constriction. This may be particularly striking in the surgical neck of the femur. The ribs may be abnormally broad and short but narrowed at the vertebral ends so that they resemble canoe paddles [11]. There may be oval radiolucencies in the tibial and distal femoral metaphyses, representing residual islands of cartilage. The first lumbar vertebra may be cuneiform. Beaking or anterior hypoplasia occurs typically in this vertebra and in T12; their posterior displacement causes a gibbus deformity. The odontoid may be quite hypoplastic. Along with macrocephaly, the large sella turcica may be omega or J-shaped (Figure 81.14). The calvaria may have a ground-glass appearance, and the mastoids may be sclerotic. Eruption of the teeth may be impaired [30, 31]. The height of the mandible may be reduced, and teeth may be displaced so far toward its inferior border that the cortex of the mandible is nearly penetrated by the roots of the teeth.

Cytoplasmic inclusions are more prominent in Maroteaux–Lamy syndrome than in any of the other mucopolysaccharidoses. They can be seen in 90–100 percent of granulocytes (Alder granules) [32] and as many as 50 percent of lymphocytes [10]. The inclusions are metachromatic. Lysosomal inclusions are also seen in Kupffer cells and in hepatocytes [33], as well as in platelets [34], and in cells of the conjunctiva, cornea, and skin [35]. Fibroblasts may contain large, clear, juxtanuclear inclusions. Large quantities of dermatan sulfate are excreted in the urine, but the total mucopolysaccharide in the urine may be normal [30]; so screening tests for urinary mucopolysaccharidosis may be misleading.

GENETICS AND PATHOGENESIS

The inheritance of this disorder is autosomal recessive. Multiple affected siblings and normal parents have been observed in a number of families, and consanguinity has been documented [10, 36]. The site of the defect is in the activity of N-acetylgalactosamine-4-sulfatase (Figure 81.1), which is coded for by a gene on chromosome 5 at position q13-14 [6].

N-acetylgalactosamine-4-sulfatase catalyzes the hydrolysis of the sulfate from moieties of N-acetylgalactosamine-4-sulfate which occur in dermatan sulfate. The moieties are also found in chondroitin-4-sulfate. Defective activity in this hydrolysis may account for some of the abnormalities in the joints, but chondroitin sulfate is not found in the urine because it can be degraded by hyaluronidase. The enzyme is also known as arylsulfatase B. The human enzyme has been purified [37, 38]. Its biosynthesis and processing involve the phosphorylation of mannose moieties and proteolysis in the classic lysosomal enzyme pattern [39, 40].

It was initially demonstrated, using artificial substrate, that this enzyme was deficient in Maroteaux–Lamy disease [6, 41–44]. The enzyme also has uridinediphospho-N-acetylgalactosamine-4-sulfate sulfohydrolyase activity [44].

cDNAs for the human [4, 5] and feline [45] enzymes have been cloned. The human monomeric protein contains 533 amino acids, including a 46 amino acid signal peptide. There is considerable homology with other sulfatases.

Correlation of clinical severity with the amount of residual enzyme activity has not been possible, but over 20 percent of normal activity is consistent with a normal phenotype [46]. Clinical manifestations have not significantly correlated with levels of urinary GAG or activity of the enzyme [18]. On the other hand, morbidity and longer survival were reported in those with unary GAG levels less than 100 μg/mg creatinine [47]. The disorder has been diagnosed prenatally by enzyme assay [48]. Heterozygosity may also be demonstrated by assay of arylsulfatase B activity [49].

Analysis of the nature of mutation promises to permit better correlation of genotype with phenotype. Southern blot analysis of genomic DNA of 17 patients revealed no deletions or rearrangements [50]. The majority of patients were compound heterozygotes for two mutations.

Homozygous deletion of a base, DG238, was found in a patient with a severe disease [9]. Severe disease was also present in a patient with different deletions on the two alleles [51]. A similar degree of severity was found in a patient with a missense conversion of cystine 117 to arginine [8], while the more conservative conversion of glycine 137 to valine was found in a patient with an intermediate phenotype [52]. Two different frameshift mutations led to stop codons in a child with severe disease [51]. Mild disease was found in a patient with two mutant alleles, one a leucine 266 to proline change, and the other a cysteine 405 to tyrosine [7]. Among 45 missense mutations in CpG dinucleotides [53] the vast majority of mutations were unique as to a small number of families.

TREATMENT

Surgical corrective procedures may be useful in the management of carpal tunnel syndrome, the hips, or the cornea. Laminectomy and removal of the thickened dura has led to improvement in myelopathy [27]. Replacement of aortic and mitral valves has been successful [14].

Bone marrow transplantation was carried out in a 13-year-old girl with normal intelligence who had advanced cardiac failure and obstructive apnea, requiring oxygen during sleep and a tracheotomy [54]. Following successful engraftment, urinary excretion of mucopolysaccharide decreased, as did hepatosplenomegaly. Cardiopulmonary function became normal. Visual acuity improved, though the cloudy appearance of the cornea was unchanged. There was no obvious change in the dysostosis multiplex. Bone marrow transplantation has also been carried out in a feline model of the syndrome in which arylsulfatase B activity was deficient [55–58]. Corneal clouding of these animals disappeared. A model in rats has also been established [59] which should facilitate experimental therapy. Needle biopsy of the liver in human transplanted patients has revealed clearing of Kupffer cells and hepatocytes of stored glycosaminoglycan [59]. Enzyme replacement therapy has been recommended to be done as soon as the diagnosis is confirmed [60]. Comprehensive guidelines on management have been developed by an international group [61]. Enzyme replacement therapy has been accepted as a safer option than hematopoietic stem cell transplantation. Naglazyme is given intravenously so it does not appear to reach poorly vascularized places, such as joints and cornea.

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82

Sly disease/β-glucuronidase deficiency/ mucopolysaccharidosis VII

MAJOR PHENOTYPIC EXPRESSION

Short stature, coarse facies; hepatosplenomegaly; kyphoscoliosis and vertebral anomalies, including odontoid hypoplasia; impaired mental development; dystosis multiplex; increased excretion of glycosaminoglycans; and deficiency of β-glucuronidase.

INTRODUCTION

In 1973, Sly and colleagues [1] reported a patient with what they recognized as a distinct mucopolysaccharidosis in whom the activity of lysosomal β-glucoronidase was deficient. Complementation studies by Quinton and colleagues [2] on fibroblasts derived from the patient had revealed this disease to be different from any previously encountered mucopolysaccharidosis. Subsequently, a small number of patients has been described. A considerable variation in clinical expression has been observed [3]. The classic infantile form is similar to Hurler disease, but much milder presentations occur. At the extreme end of the spectrum is the acute fetal or neonatal form characterized by hydrops fetalis [4].

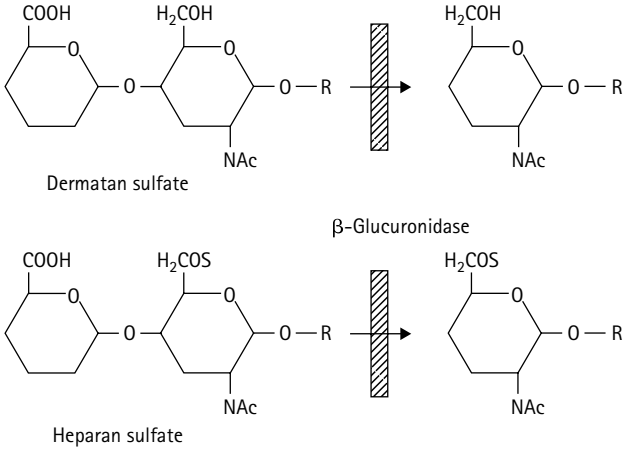

Figure 82.1 The β-glucuronidase reaction. In addition to the removal of glucuronic acid residues from dermatan sulfate and heparan sulfate, the enzyme catalyzes this reaction with the chondroitin sulfates.

Mucopolysaccharidurıa in this condition may be mild. The defective enzyme (Figure 82.1), β-glucuronidase [1], catalyzes the removal of glucuronic acid residues that occur in dermatan sulfate and heparan sulfate; it is also active against chondroitin 4- or 6-sulfates, but these compounds are not stored or excreted in the urine, because of the activity of hyaluronidase [5].

β-Glucuronidase was a key enzyme in the development of current understandings of lysosomal enzyme processing. It was in studies of this enzyme that the mannose-6-phosphate recognition marker was first identified [6]. Full-length cDNAs from the human and rodent genes have been cloned, sequenced, and expressed; they encode polypeptides of 651 and 648 amino acids, respectively [7, 8]. The gene is 21 kb in length and contains 12 exons [9]. It is located at chromosome 7 q21-22 [10]. The mutation has been defined in the initial patient [11] and a small number of others [11–13].

CLINICAL ABNORMALITIES

The original patient [1] was characterized by shortness of stature, relatively severe skeletal abnormalities as compared with other mucopolysaccharidoses, and relatively mild impairment of cognitive function. He was first seen at seven weeks for metatarsus adductus and recognized as having unusual facial features. The nasal bridge was depressed, the nostrils were anteverted, the maxillae prominent, and the eyes were wide and had epicanthal folds. The abdomen was protuberant and the liver palpable 4 cm below the costal margin. The spleen was at 3 cm. There was a long, diastasis recti and an umbilical hernia. There was puffy skin over the dorsa of the hands and feet. A thoracolumbar gibbus had already developed. Short stature had been evident at 18 months, and the head circumference reached the 98th percentile by five months. The gibbus increased, and he developed a pigeon breast with a sharp angle between the body of the sternum pointing forward and the xyphoid pointing backward. He developed bilateral inguinal hernias. Hepatomegaly increased.

Developmental milestones and neurologic examination were normal for two years. By three years, impaired development, especially in speech, was evident, but it appeared to be nonprogressive. Orthopedic problems progressed and walking became painful. He died suddenly at 20 years of age, but autopsy did not reveal the cause – a relatively common occurrence in patients with odontoid hypoplasia and other problems about the neck.

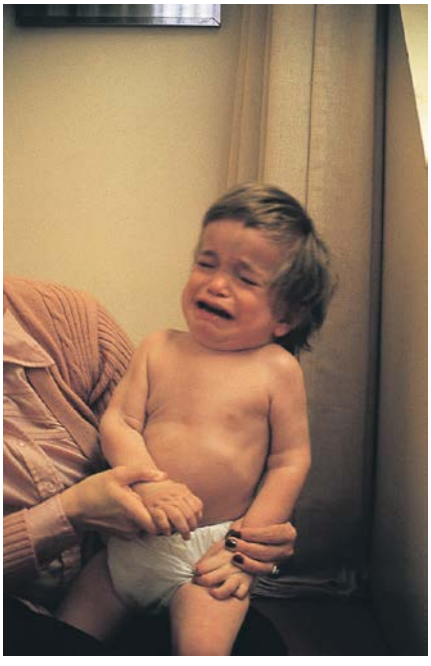

Figure 82.2 TM: A boy with mucopolysaccharidosis VII had the classic phenotype. Facial features were coarse. He had hepatosplenomegaly, a gibbus and bilateral inguinal hernia, and developed hydrocephalus [16]. (This illustration was kindly provided by Dr Kenneth Lyons Jones of UCSD.)

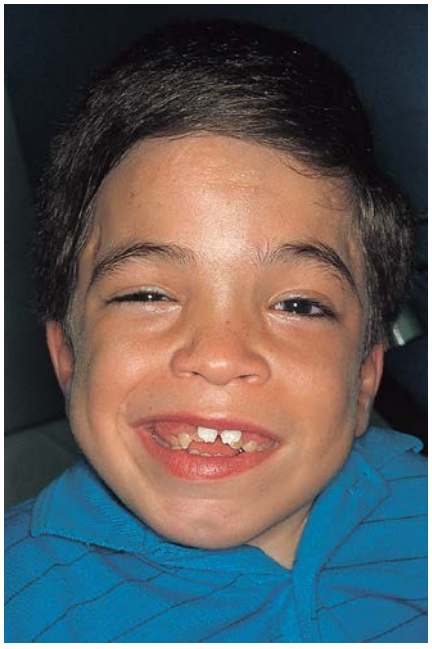

Figure 82.3 TM: With time the features were coarser. There was marked gingival hyperplasia. He died at 15 years of age. (This illustration was kindly provided by Dr Kenneth Lyons Jones of UCSD.)

This classic presentation [1, 14–19] of a moderately severe Hurler-like mucopolysaccharidosis with modest impaired mental development (Figures 82.2 and 82.3) represents a relatively uncommon intermediate presentation of MPS VII. There are appreciably milder forms, and the most severe prenatal or neonatal forms appear to be the most common. Clouding of the cornea became evident in the index patient by eight years of age, but in others it has been evident earlier. It can usually be readily demonstrated by slit lamp examination.

Most patients have had frequent upper respiratory infections, and pneumonia has occurred in some. Hernias, shortness of stature [1], relative macrocephaly, and coarse features are regularly observed. Most have had gingival hyperplasia. Gibbus deformity has regularly been reported.

Joint contractures have been observed and also hydrocephalus [16], concomitants of a classic mucopolysaccharidosis. Some have had dislocated hips [18]. Camptodactyly has been noted at birth along with absence of distal phalangeal creases, indicating prenatal onset. All have had hepatosplenomegaly. Developmental delay has been mild to moderate.

A severe example of this phenotype [3] died at 2½ years after a course characterized by marked inhibition of growth, hepatosplenomegaly of neonatal onset, corneal clouding, by seven months, and gingival hypertrophy. Icterus, recurrent diarrhea, and hypoalbuminemia may have been unrelated consequences of giant cell hepatitis and carbohydrate intolerance.

The most severe phenotype, a neonatal or fetal form [4, 20–24], is typified by nonimmune hydrops fetalis (Figure 82.4). Hydrops fetalis was diagnosed prenatally [25]

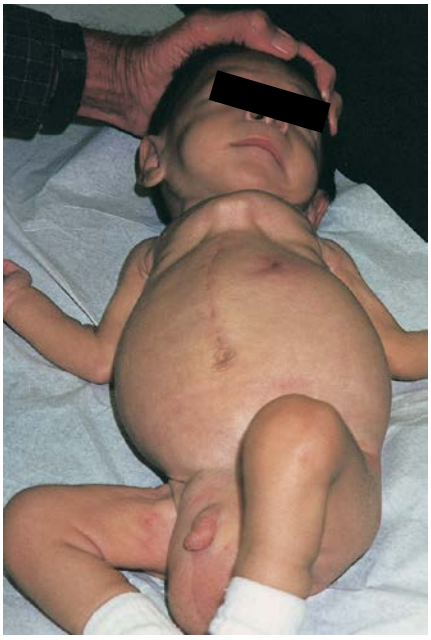

Figure 82.4 WM: A 6-week-old infant with Sly disease. He had fetal and neonatal ascites evident at birth and macrocephaly. There was prominent subcutaneous tissue visible in the nares. Liver and spleen were enlarged, and he had bilateral inguinal hernias. He had a gibbus and a prominent manubrium.

in a patient who developed hypertrophic cardiomyopathy. Recurrent fetal hydrops was reported [26]. The metabolic differential diagnosis of hydrops fetus is shown in the Appendix. Three reports were of fetal death, and family histories of hydropic or neonatal patients indicate an increase in spontaneous abortions [24]. This is a distinct presentation for a mucopolysaccharidosis recognizable *in utero* or at least at birth. Dystosis multiplex is present at birth in these patients. The facies is coarse. There is pitting neonatal edema, ascites, and hepatosplenomegaly. Talipes equinovarus has been reported and congenital dislocation of the hip [4]. Cardiomyopathy may be progressive [4]. Death may occur in the first six months.

A number of patients have also been reported with milder manifestations with onset after four years of age or much later and with skeletal manifestations predominating [5, 17, 24, 27–29]. One was 14-years-old at the time of report [3] and was well except for hypertension and fibromuscular dysplasia causing narrowing of the aorta and femoral arteries. Height was normal, and dysostosis multiplex of ribs and spine was very mild. Speech therapy was required at three years. Another patient [28] appeared normal at 11 years, except for bilateral club feet, which had been surgically corrected, and frequent upper respiratory infections in childhood. Intelligence was tested as normal. A 13-year-old girl [29] had normal height, moderately impaired mental development, a short neck and protruding sternum, corneal clouding, dysplastic hips, and vertebral abnormalities.

Detailed follow up of this patient, at which time she was the longest known survivor, was published [30] at 37 years of age; she died unexpectedly that year. She had spastic tetraplegia, odontoid hypoplasia, and narrowed intervertebral foramina in the cervical spine. Surgery relieved cord compression. There was no hepatosplenomegaly. Another variant was described [31] as an oligosymptomatic 20-year-old male despite severe skeletal dysplasia. It is clear from these observations that there is a very wide spectrum of clinical phenotypes.

Dysostosis multiplex (Chapter 76) has been present in roentgenograms of patients with β-glucuronidase deficiency, especially those with the classic and neonatal forms. The skull is large and the sella J-shaped. The ribs are spatulate [1, 3, 16]. Vertebrae are shortened and anteriorly beaked, and there may be odontoid hypoplasia [1, 3, 16]. Dysplasia of the hips is associated with hypoplastic ilia [1, 3, 16, 18]. Proximal metacarpals are pointed [1, 16].

Coarse lamellar Alder-Reilly inclusions are seen in peripheral granulocytes [1, 31,32] and also in the bone marrow. Pathologic examination has revealed vacuolated hepatocytes; electron microscopy has shown cytoplasmic membrane-bound vesicles [18]. The stored material stains with alcian blue, and this staining may be seen in cultured fibroblasts, which also display metachromasia.

Glycosaminoglycan excretion is usually moderately increased in this condition [1, 3, 18], but screening tests for mucopolysaccharide excretion may be normal [16], and some adult patients have not had increased glycosaminoglycan excretion. The material has been shown to consist of dermatan sulfate and heparan sulfate [3, 18].

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion via mutant genes on chromosome 7 [10]. Its incidence has been estimated at one in 300,000 live births in British Columbia [33]. The molecular defect is in the enzyme β-glucuronidase. (EC 3.2.1.31) [1, 34]. Cultured fibroblasts from patients accumulate sulfated mucopolysaccharide when incubated with $^{35}SO_4$, and this abnormality is corrected by the addition of bovine liver β-glucuronidase to the medium [34]. Identity of the corrective factor and the glucuronidase was demonstrated by coelectrophoresis in polyacrylamide gel. Virtually complete deficiency has been demonstrated with a variety of synthetic substrates in leukocytes and in fibroblasts [34]. It has also been detected in serum [18]. The enzyme is a tetramer of 75 kDa subunits [35]. It is synthesized as a precursor protein and processed at the carboxyl end by the loss of the signal peptide [34]. Immunochemical studies have indicated the presence of cross-reacting material (CRM) in patients with the disease [36]. The measurement of enzyme activity has not correlated well with the degree of severity of phenotype.

Reduced levels of enzyme were found in the leukocytes of parents [1]. Prenatal diagnosis is available by the assay of cultured amniocytes or chorionic villus material. In

families in which the mutation is known, this is the method of choice for prenatal diagnosis and for carrier detection.

The human and murine gene has considerable homology in the coding region for the mature protein. Alternate splicing of the human gene leads to two types of cDNA [9], the shorter one containing a large deletion in exon 6. A pseudodeficiency allele was defined in the study of a pseudodeficient mother of a child who carried a mutation, L176 F [37]. The mother had greatly reduced levels of β-glucuronidase without evident clinical effect and a substitution of asparagine for aspartic acid, D152N. The existence of pseudodeficiency greatly complicates prenatal diagnosis by enzyme assay and also heterozygote detection [38].

The L176F mutation was also found in a Brazilian patient who attended a special school at 18 years [39]. The mouse model of this common mutation had low levels of activity and a milder phenotype.

In the index patient, there was a compound of two alleles, a missense tryptophan 627 to cysteine and a nonsense arginine 256 to stop [11]. In two Japanese patients, mutations at two CpG sites have been identified: alanine 619 to valine [12] and arginine 382 to cysteine [13]. Four more mutations described in two Caucasian patients [40] were a 38 bp deletion at positions 1642–1679 in exon 10 caused by a single base change that generated a new splice site; and three point mutations – proline 148 to serine, tyrosine to cysteine, and tryptophan 507 to a stop-codon. A prenatally diagnosed patient with hydrops fetalis [41] was found [13] to have a C-to-T transition that led to a substitution of cysteine for arginine 382. In studies of 21 patients with hydrops fetalis or early severe disease [42], 19 different mutations were reported. By 2002, 45 different mutations had been reported [43], some 90 percent of them point mutations. By 2009, information was summarized on 49 unique disease-causing mutations [44]; missense mutations accounted for 79 percent.

The mechanism for shortened bones in this disease was studied in the MPS VII mice [45]. Accumulation of chondroitin-4-sulfate in growth plates led to reduced expression of leukemia inhibiting factor (LIF) and reduced tyrosine phosphorylation of a transcription factor.

TREATMENT

Specific treatment such as bone marrow transplantation (BMT) was successful in neonatal mice, as contrasted with adult mice with β-glucuronidase deficiency [46]. Enzyme replacement initiated at birth followed by BMT at five weeks was highly successful in this model. This deficiency has also been found in a dog model [47], which has been reported to more closely mimic the human clinical disease and enzyme deficiency than the mouse, in which activity is 20 percent of control. The availability of animals should be useful for the development of gene transfer. Among approaches to gene therapy, affected murine fibroblasts were transfected with a retroviral vector containing human β-glucuronidase cDNA and implanted into mice; there was expression of enzyme *in vivo* and disappearance of lysosomal storage in the liver and spleen [48]. In a Brazilian patient, bone marrow transplantation led to fatal complications [39].

Supportive treatment should include attention to potential cervical instability – for example, during anesthesia. Corneal transplantation may be useful in older patients in whom vision is impaired. Physiotherapy is useful for joint stiffness and the preservation of function.

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PART 10

MUCOLIPIDOSIS

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83

Mucolipidosis II and III/ (I-cell disease and pseudo-Hurler polydystrophy) N-acetyl-glucosaminyl-l-phosphotransferase deficiency

MAJOR PHENOTYPIC EXPRESSION

Coarse features, shortness of stature, malocclusion, gingival hypertrophy, progressive developmental impairment, pain and limitation of joint motion, dysostosis multiplex cytoplasmic inclusions in fibroblasts (Figure 83.1), deficient intracellular activity of many hydrolases, and elevated activity of these enzymes in serum because of defective post-translational modification of acid hydrolases, a consequence of the fundamental defect in N-acetylglucosaminyl-(GlcNAc) 1-phosphotransferase.

INTRODUCTION

Mucolipidosis II and III reflect multiple deficiencies of many lysosomal hydrolases that require post-translational processing to form the recognition site that permits their cellular uptake. The fundamental defect is in N-acetylglucosaminyl-l-phosphotransferase (GlcNAc phosphotransferase) (Figure 83.2) [1]. The lysosomal enzyme substrates for this enzyme are glycoproteins containing reactive mannose molecules, and in the reaction a GlcNAc phosphate is linked to the mannose; a subsequent phosphodiesterase reaction cleaves off the GlcNAc, leaving the mannose phosphate recognition site. Patients with I-cell disease, or mucolipidosis II, have complete deficiency of this enzyme, while patients with mucolipidosis III have varying amounts of residual activity of the enzyme. Variable patterns of clinical phenotype in mucolipidosis III reflect the considerable variation in enzyme activity as well as its effect on so very many lysosomal enzymes. The extent of the phenotypic variability has doubtless not yet been defined. Leroy and colleagues [2, 3] gave the disease its name I-cell disease, the I indicating inclusions.

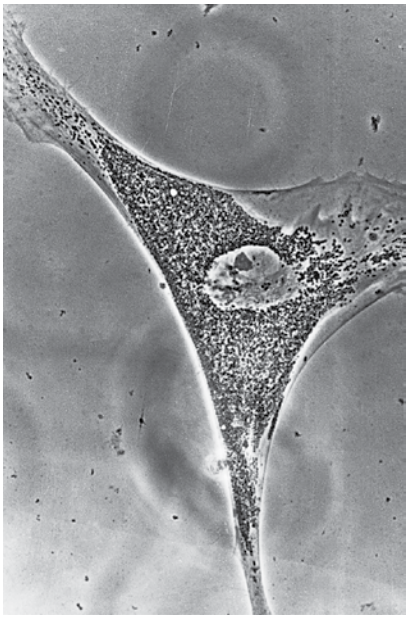

Figure 83.1 I-cell in fibroblast culture illustrating the characteristic cytoplasmic inclusions. Courtesy of Dr. Jules Leroy State University of Antwerp, Belgium.

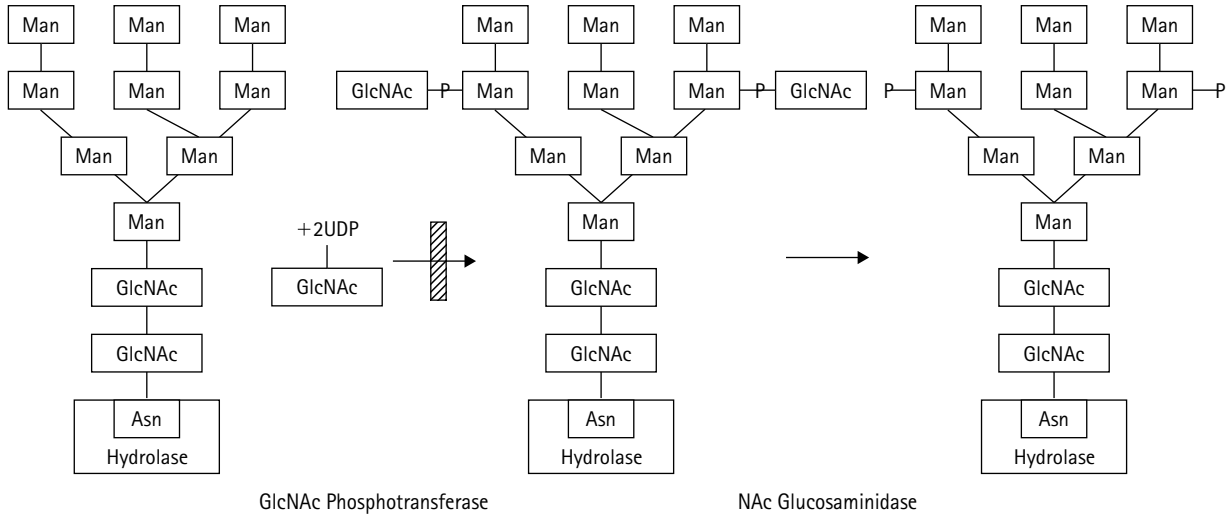

Figure 83.2 (N-acetylglucosamine [GlcNAc] phosphotransferase, the site of the defect in I-cell disease and in mucolipidoses III. The pathway for phosphorylating the acid hydrolase enzymes is shown as a two-step reaction, which ultimately forms the mannose-6-phosphate recognition site that targets the enzyme for cellular uptake. Abbreviations employed in addition to GINAc: UDP-GlcNAc for uridine diphosphate-GlcNAc; Man, mannose; and Asn to indicate the linkage of the oligosaccharide to an asparagine residue of the enzyme protein.

There is genetic heterogeneity in mucolipidosis III caused by the presence of two genes which code for the three subunits of GlcNAc phosphotransferase, α/β and γ [4, 5]. Abnormalities in both genes have been found in different patients with mucolipidosis III. In the absence of phosphorylation of mannose, trafficking of lysosomal hydrolase enzymes is impaired.

CLINICAL ABNORMALITIES

Mucolipidosis II/III shares many of the clinical manifestations of the classic mucopolysaccharidoses. In fact, the roentgenographic characteristics are those of a florid dysostosis multiplex (Figures 83.3–83.8). The films of one of our patients [6] were kept in the teaching file of a medical school department of radiology as exemplifying Hurler disease. The disease was originally described [7] as pseudo-Hurler polydystrophy. There is, however, no mucopolysaccharidiuria. The long bones are short and thick. The distal radius and ulna tilt toward each other. The proximal phalanges are bullet shaped and the metacarpals are broad distally and pointed proximally. The ribs are broad and spatulate. Vertebral bodies are short and Ll and T12 may be anteriorly beaked (Figures 83.6 and 83.7). There may be early craniosynostosis (Figure 83.8). In other patients, the skull may be normal. There may be hypoplasia of the odontoid. Degenerative changes of the joints, especially the proximal femoral areas, may be characteristic.

Patients usually present between two and four years of age with symptoms referable to the joints [6]. Pain is severe enough to awaken them from sleep. Tenderness and early progressive stiffness and limitation of motion may lead to a presumptive diagnosis of juvenile rheumatoid arthritis,

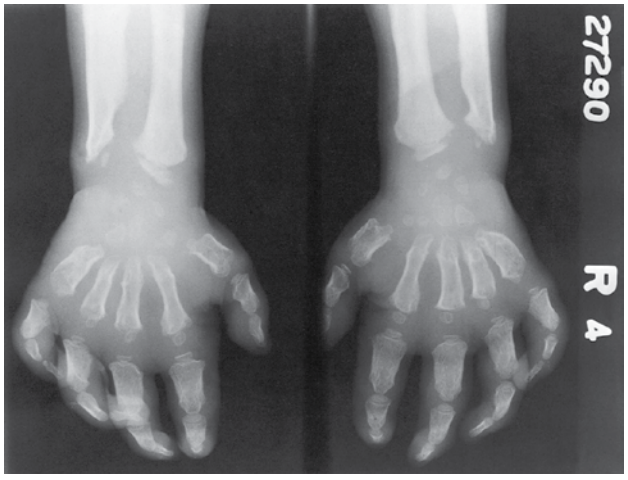

Figure 83.3 Roentgenogram of the hand of an 11-year-old patient with mucolipidosis type III. The picture was that of an extreme degree of dysostosis multiplex.

but the sedimentation rate remains normal [8]. All the joints may be involved, and most patients develop some contractures. The claw hand that results by six years of age may be indistinguishable from that of the patient with Hurler disease (Figure 83.9). In some patients, the hands may be quite different, with prominent joints but elongated digits without contracture (Figure 83.10). A carpal tunnel syndrome may develop. Contractures of the knees, hips, and elbows are in flexion, leading to a jockey-like appearance (Figure 83.11). These changes lead regularly to shortness of stature (Figure 83.10), but this may be variable. Females may be taller and less severely affected than males [9, 10]. Progressive destructive changes in the hip may lead to a waddling gait and compromised mobility. Rarely, isolated

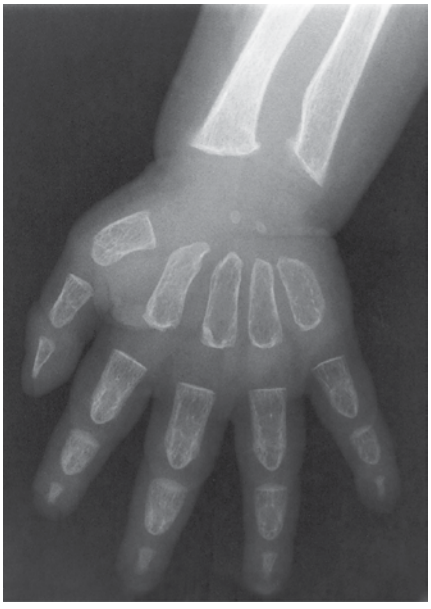

Figure 83.4 Roentgenogram of hand of a ten-month-old infant with mucolipidosis III. There was extreme osteopenia with a fine inner reticular pattern. The phalanges were bullet shaped. The metacarpals were broad at their distal ends and tapered proximally. The radius and ulna were angulated toward each other.

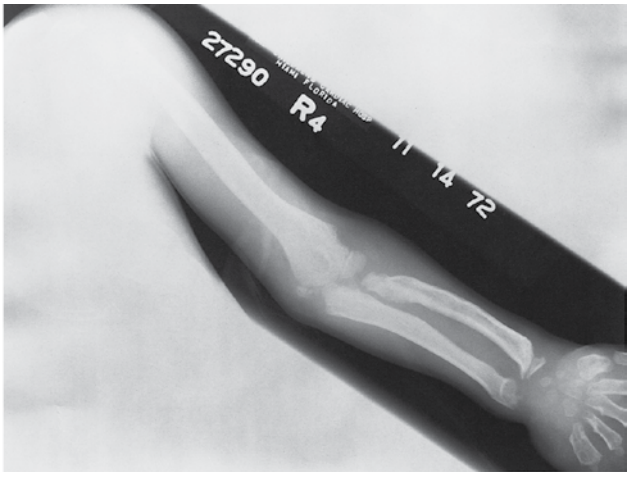

Figure 83.5 Roentgenogram of the radius and ulna of a patient with mucolipidosis III.

involvement of the hip and spine may be the only clinical manifestations [11]. Late effects are destruction of the femoral heads and of vertebral bodies.

The facial features may also be sufficiently coarse to suggest a diagnosis of mucopolysaccharidosis (Figures 83.12–83.16), but in some patients the face may appear normal (Figure 83.9). Hirsutism may be prominent and there may be synophris. The appearance of the mouth may be characteristic, with gingival hypertrophy (Figure 83.16) and crowding of teeth with malocclusion (Figure 83.17). Gingival hypertrophy is always seen in I-cell disease (mucolipidosis II). The skin may become thickened. The

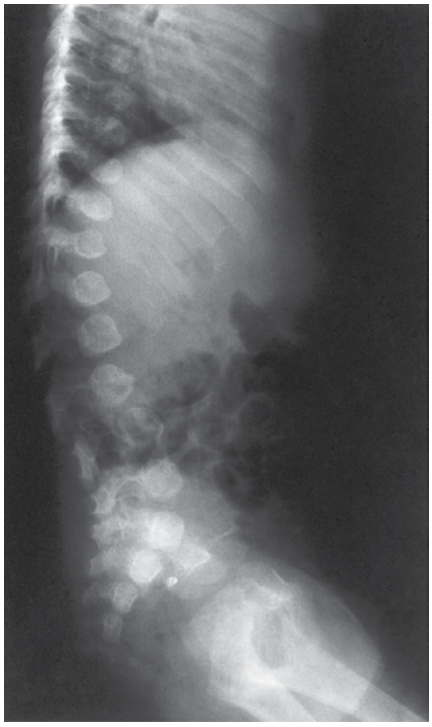

Figure 83.6 Roentgenographic appearance of the broad spatulate ribs and of the spine of a patient with mucolipidosis III.

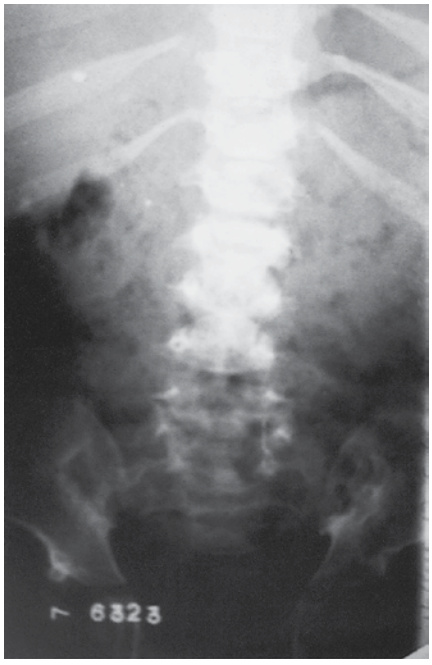

Figure 83.7 Roentgenographic appearances of the broad spatulate ribs and of the spine of another patient with mucolipidosis III.

corneas may appear normal, but steaminess of the cornea may require a slit lamp for visualization, or it may be evident to the naked eye, as in Hurler or Maroteaux–Lamy syndromes. In some, slit lamp examination may be normal. Hyperopic astigmatism and mild retinopathy have also

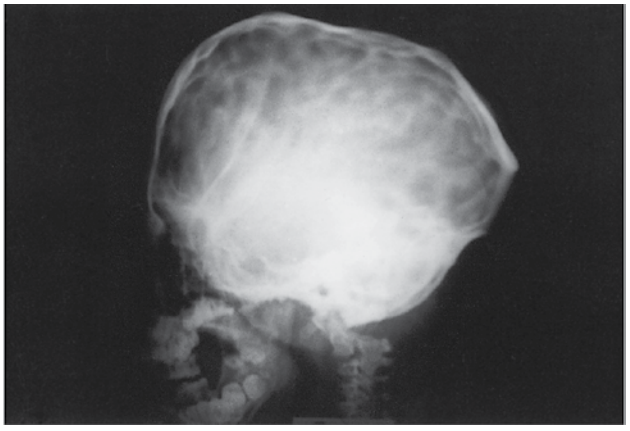

Figure 83.8 Roentgenogram of the skull of the patient in Figure 83.6 illustrates the shape and beaten silver appearance of premature craniosynostosis.

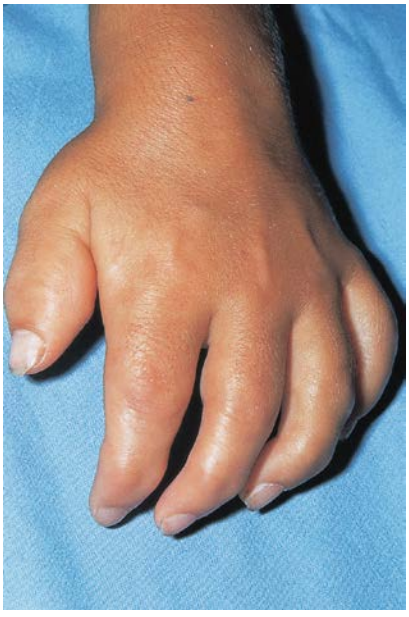

Figure 83.9 The claw-hand deformity may be identical to that of Hurler syndrome.

Figure 83.10 The hand of the infant shows periarticular swelling and limitation of joint motion.

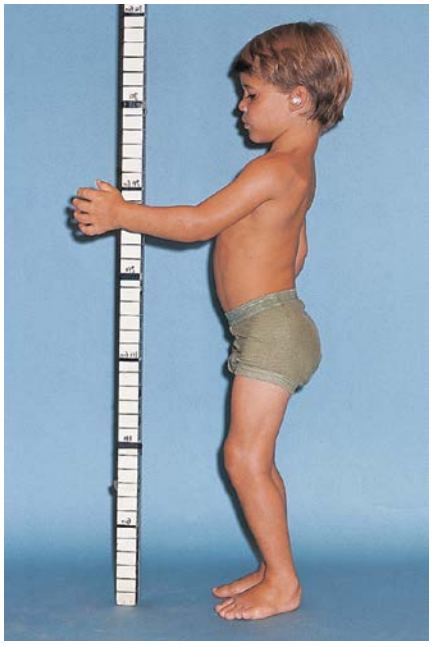

Figure 83.11 An 11-year-old boy with mucolipidosis III. Contractures of the knees and hips and exaggerated lordosis give a bent position.

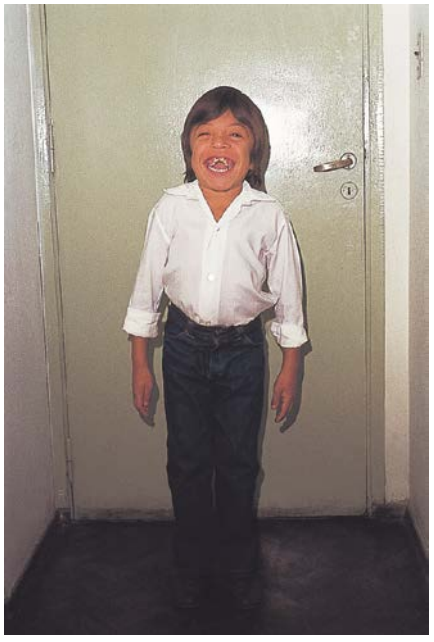

Figure 83.12 A ten-year-old boy with mucolipidosis was short and had limitation of motion at the elbows, knees, and hands.

been described [12]. Intelligence may be normal [6], but most patients with mucolipidosis III have some limitation in cognitive function. IQ levels of 70–90 are commonly encountered. In contrast, patients with I-cell disease have degrees of impairement incompatible with walking or talking. Infiltration of the endocardium may lead to aortic regurgitation and its characteristic diastolic murmur by the end of the first decade, but symptoms of cardiac insufficiency

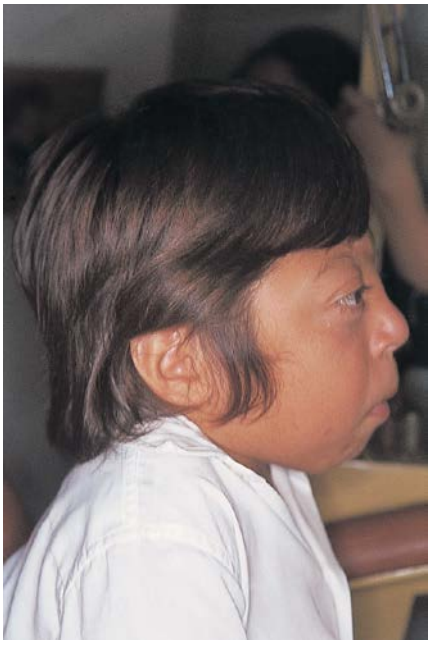

Figure 83.13 Facial features of this patient were coarse and the eyes prominent.

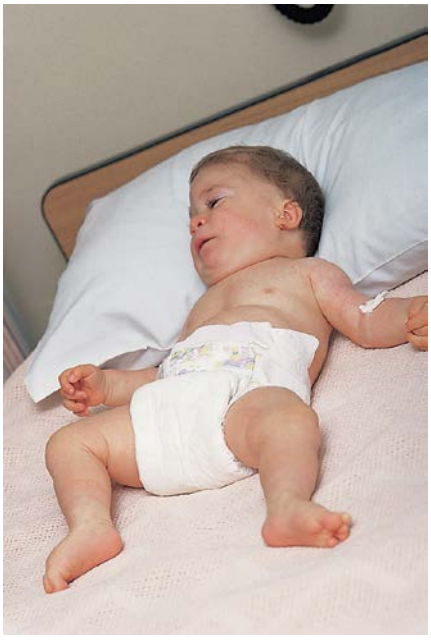

Figure 83.14 RV: A one-month-old infant with mucolipidosis III. She was developmentally delayed and had coarse features. The position of the legs was in treatment of bilateral dislocations of the hips.

are rare. The liver is only moderately enlarged [13]. Some patients have inguinal hernias. Intelligence is usually to some extent compromised, but not severely [6, 11]. Life expectancy is appreciably better than in mucolipidosis II, and survival to adulthood is not uncommon [11, 14].

The histologic characteristic of these mucolipidoses is the appearance of cytoplasmic inclusion bodies. These dense

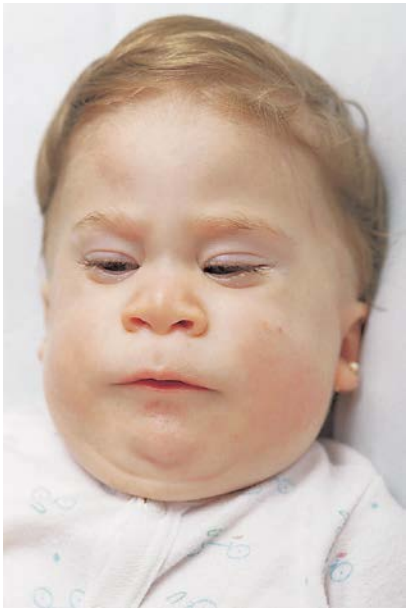

Figure 83.15 Close up of the face illustrates the coarse features indicative of storage of mucopolysaccharide within the skin, illustrated for example in the nose.

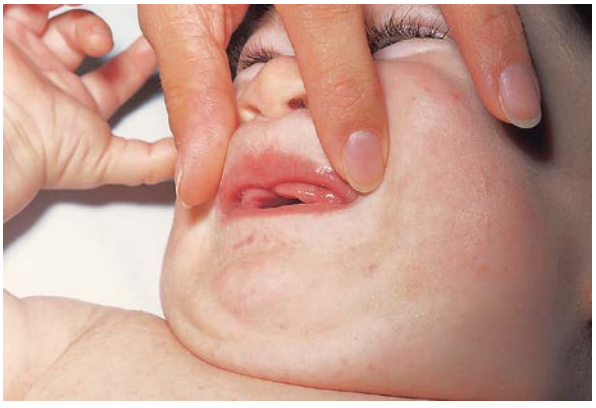

Figure 83.16 The hyperplasia of the gums was well delineated by ten months; it had been noted by the parents at birth. The gingiva was also cleft.

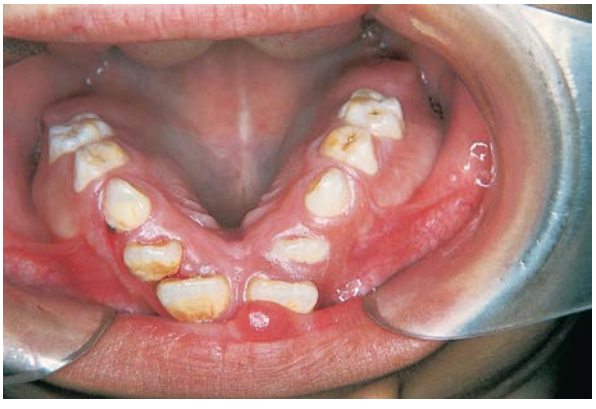

Figure 83.17 Gingival hypertrophy and malocclusion were prominent features in this patient.

bodies, seen in cultured fibroblasts on phase microscopy, are the inclusions that gave I-cell disease its first name (Figure 83.1). Inclusions or vacuolation may be seen in other cells, such as biopsied cornea, bone marrow cells, or lymphocytes [15, 16]. These patients do not have excessive excretion of mucopolysaccharides in the urine.

Clinical features of patients with defects limited to the γ-subunit tend to be somewhat milder. Recognition of symptoms may be in the latter half of the first decade of life [17, 18], and generally there is normal psychomotor development. The problems are mostly orthopedic, including dysostosis, joint stiffness, and joint pain.

GENETICS AND PATHOGENESIS

Specific biochemical diagnosis is often first suggested when fibroblasts or lymphocytes are assayed for the activity of lysosomal hydrolase enzymes. Defective activity is demonstrable for a number of different enzymes, such as hexosaminidase, glucuronidase, and arylsulfatase A [10]. Activities of the same enzymes are high in the media in which the cells are grown [19], which suggested at first that the cells were leaky. These same enzymes may be found in high levels of activity in the serum of patients. Activities may be 100-fold the normal level for some enzymes. The reason for this is not leaky cells but abnormal lysosomal enzymes, which are normally secreted and then avidly taken up. Mucolipidosis enzymes cannot be taken up by normal cells while enzymes from normal cells are taken up normally by mucolipidosis cells [20]. This is because normal enzymes have the mannose-6-phosphate recognition marker that is essential for normal transport of the enzyme, and those of mucolipidosis patients are deficient in this phosphomannosyl signal. Activities of β-glucosidase in fibroblasts of patients are normal, because this enzyme is targeted to lysosomes by a phosphorylation-independent mechanism [21].

The enzyme that catalyzes the initial phosphorylation of mannose residues in the glycoprotein enzymes is formally UDP-N-acetylglucosamine: lysosomal enzyme-N-acetylglucosamine-l-phosphotransferase; because its other substrate is UDP-N-acetylglucosamine, we have shortened this to GlcNAc phosphotransferase. Somatic cell hybridization studies have revealed distinct complementation groups [22, 23], which are now referred to as groups A, B, and C. Group A is the most common; many I-cell patients also fit into this group [24]. Group C is uncommon and group B is rare. In the phosphotransferase assay, which utilizes α-methylmannoside as acceptor, patients with mucolipidosis III in the complementation group C have normal enzyme activity [25, 26]. Those of groups A and B display defective activity against all substrates. The enzyme normally has two distinct functions: recognition of and affinity for the lysosomal enzyme protein, and catalytic phosphorylation of mannose residues. In parallel, studies with lysosomal enzymes as substrates and methylmannoside substrate have elucidated the existence of two distinct groups of patients: one in which activity against both is deficient, and the other in which activity against α-methylmannoside is normal but phosphorylation of lysosomal enzymes is impaired. This would be consistent with specific interference with recognition versus defect in catalytic function [27]. In 2008, Cathey *et al.* [28] proposed an updated nomenclature system, replacing the term ML II with ML II alpha/beta, ML IIIA with ML III alpha/beta, and ML IIIC with ML III/gamma.

The α/β gene has been mapped to chromosome 12p23.2 [4] and is identified as *GNPTAB*. The γ gene has been mapped to 16p13.3 [5] and is labeled *GNPTG*. Some patients with mucolipidosis III have been found to have abnormalities in the α/β gene, because there is reduced transcription to mRNA [4]. In other families with mucolipidosis III, a mutation was found in the γ gene [5]. This insertion of a cytosine at codon 167 leads to a frameshift and a premature termination 107 bp downstream [3]. There are more than 126 known mutations in *GNPTAB*, of which some 38 are publicly listed and more than 29 mutations in *GNPTG*, of which eight are publicly listed [29].

In the Saguenay–Lac-Sainte-Jean population of Quebec, Canada the frequency of this disease is the highest in the world. A single mutation c.3503-3504 delTC was found in all obligate carriers [30]. This would be consistent with a founder effect. Eleven mutations were found in 13 patients, eight of them novel. In the *GNPTG* gene, two mutations were c.610-G>T and c.639delT [31].

Genetic transmission is autosomal recessive. Consanguinity has been observed [9]. Heterozygotes have been reported to have intermediate levels of GlcNAc phosphotransferase in leukocytes and cultured fibroblasts [25]. Prenatal diagnosis of I cell disease has been accomplished by analysis of amniotic fluid [32], and chorionic villus [33]. Pacman dysplasia, a lethal skeletal disease with epiphyseal stippling, has been detected prenatally [34]. In atypical patients, I cell disease was documented suggesting that Pacman patients have I cell disease.

TREATMENT

Supportive orthopedic management and physiotherapy may be useful, especially for abnormalities in the hips. It is recommended that hip surgery is delayed until after puberty. Surgical correction of carpal tunnel syndrome is useful. Intravenous pamidronate has been reported [35] to reduce bone pain and improve mobility, but biochemical, histologic, and roentgenographic evidence of bone resorption did not improve. AAV8 mediated expression of N-acetylglucosamine-1-phosphotransferase attenuated bone loss in a mouse model of mucolipidoses II. A recombinant adeno-associated viral vector was found to increase bone density significantly in a knockout mouse model [36].

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PART 11

DISORDERS OF CHOLESTEROL AND NEUTRAL LIPID METABOLISM

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84

Familial hypercholesterolemia

MAJOR PHENOTYPIC EXPRESSION

Xanthomas, coronary artery disease, hypercholesterolemia, elevated concentration of low-density lipoprotein (LDL) cholesterol in plasma, and defective activity of LDL receptor.

INTRODUCTION

Familial hypercholesterolemia (FH) is an important model disease. The fundamental defect is an abnormality in a receptor molecule [1]. The study of this disease, especially in the homozygous form, has provided insights into the regulation of the metabolism of cholesterol. This has led to practical approaches to the management of the more common heterozygous disease and other forms of hypercholesterolemia. FH makes for compelling evidence of the causal relationship between elevated levels of cholesterol in the blood and coronary atherosclerosis.

The disease is dominantly expressed in heterozygotes, who develop coronary artery disease after the age of 30 years. In homozygotes, the concentrations of cholesterol in the blood are enormous. Coronary artery disease develops in childhood. The genetics were worked out by Khachadurian [2] in Lebanon, where an unusual number of homozygotes and a very high incidence of consanguinity have been observed. Variation at a single gene locus leads to three distinct phenotypes: homozygous affected, heterozygous, and homozygous normal.

FH is heterogeneous genetically. It is caused by mutations in at least three different genes. The most common variant, accounting for approximately 93 percent of patients, is caused by mutations in the low-density lipoprotein receptor (LDLR); the resultant disease is currently known as familial hypercholesterolemia (FH). Mutations in apolipoprotein B-100 (APOB) account for approximately 5.5 percent of patients, and this disease is referred to as familial defective APOB (FDB). In approximately 2 percent of patients, the mutation is in the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*) [3]. Some 1741 mutations have been identified in the gene *LDLR*. Of these, 108 variants were found in Chinese patients [4].

The relationship between FH and LDL was established by the studies of Gofman *et al.* [5] and of Frederickson *et al.* [6]. The nature of the fundamental defect in the receptor, its variety, and the nature of mutation have been laid out in the elegant work of Brown and Goldstein [1, 7] (Figure 84.1). LDL cholesterol is taken up by cells after binding of the LDL to its receptor in coated pits on the cell surface, which then undergo endocytic internalization. When the receptor is defective, LDL cholesterol cannot be removed from the plasma, levels are very high, and the clinical consequences ensue.

Five types of defects have been established:

- In the most common, class 1, no immunoprecipitable (cross-reacting material [CRM]) receptor protein is found.
- In class 2, the protein cannot be transported to the endoplasmic reticulum and the Golgi complex.
- In class 3, the receptor does not properly bind LDL.
- In class 4, the receptor does not cluster in the coated pits and bound LDL is not internalized.
- In class 5, the receptors bind and internalize in coated pits but are unable to release the LDL in the endosome and recycle it.

FH is caused by mutations in the *LDLR* gene. The gene has been mapped to the short arm of chromosome 19, at p13.1-13.3. A very large number and variety of mutations

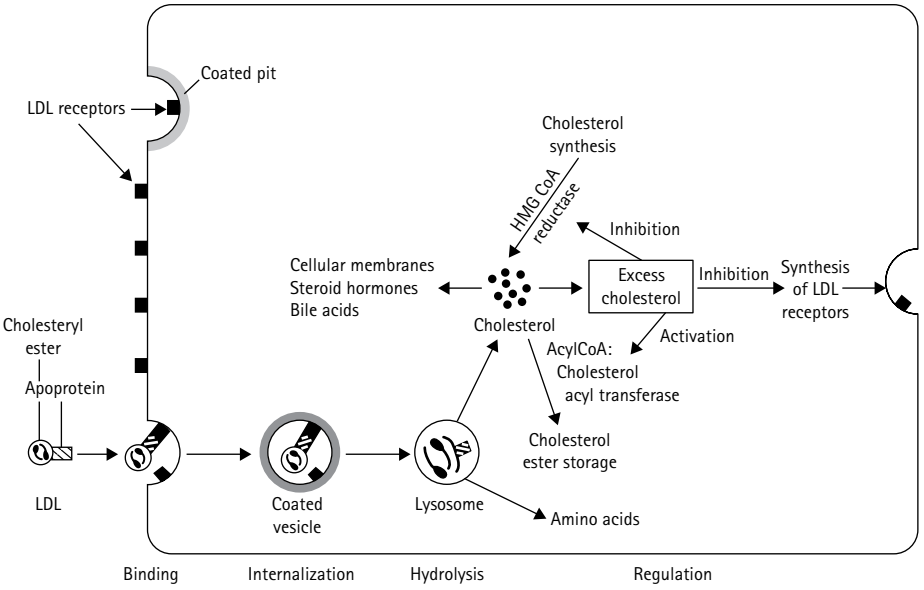

Figure 84.1 The low-density lipoprotein (LDL) receptor and its role in the regulation of the metabolism of cholesterol.

have been defined [8], and there are now two websites (www.ucl.ac.uk/fh/ and www.umd.necker.fr) [9–11]. The most common, class 1 mutations (Table 84.1) are null alleles in the sense that there is no immunoprecipitable receptor protein [12]. Mutations have been defined in each of the classes. Homozygous individuals may have two identical mutant alleles, or they may be compound heterozygotes, who inherited a different allele from each parent.

FH is also caused by the ligand defective apolipoprotein B (APOB) and by mutation in the *PCSK9* gene (HCHOLA3) [3] and *LDLRAP1*.

Individuals with the *LDLR* mutation lvs14+G-A may have a phenotype altered by SNP+$APOA_2$ gene, a SNP in the $EPHX_2$ gene or a SNP in the *GHR* gene. A SNP in intron 17 of the $1TIH_4$ gene has been associated with hypercholesterolemia in Japanese.

CLINICAL ABNORMALITIES

Homozygous familial hypercholesterolemia

Hypercholesterolemia is present from birth in heterozygotes, as well as homozygotes, but homozygotes have severe hypercholesterolemic disease [13]. Clinical manifestations appear in the first two decades of life [14]. Their cholesterol concentrations range from 600 to 1200 mg/dL (15–30 μmol/L) [15]. The first clinical manifestation is usually the appearance of xanthomas (Figures 84.2–84.7). Xanthomas may be flat (planar), tuberous, or tendinous.

Xanthomatous deposits are particularly common over the Achilles tendon and the extensor tendons of the hands. Tuberous xanthomas are seen over the elbows, knees, and elsewhere. Subperiosteal xanthomas may be seen below the knee at the tibial tuberosity and at the elbow. Trauma appears to influence the local occurrence of these lesions. Cutaneous xanthomas may be bright orange or yellow and they are prominent over the buttocks and the hands. The interdigital web between the first and second fingers is a favorite site. Xanthomas sometimes occur on the tongue or the buccal mucosa. An arcus about the cornea is regularly seen in homozygotes prior to the age of ten years (Figure 84.8).

The most significant clinical consequence of FH is the occurrence of severe atherosclerosis in the aorta and coronary arteries because LDL-derived cholesterol is also deposited in arterial atheromatous plaques [16]. Peripheral and cerebral vessels are also involved. Plaques contain abundant deposits of lipid in the extracellular space and in large foamy cells. Disease of the heart tends to be rapidly

Table 84.1 Classes of mutation of the low-density lipoprotein (LDL) receptor

Class	Defect	Binding of LDL	Internalization
1	Synthesis	Absent	–
2	Transport to Golgi	Absent or reduced	Normal
3	Binding of LDL	Reduced or absent	Normal
4	Clustering in coated pits	Normal	Defective
5	Discharge in endosome (recycling)	Normal	Normal

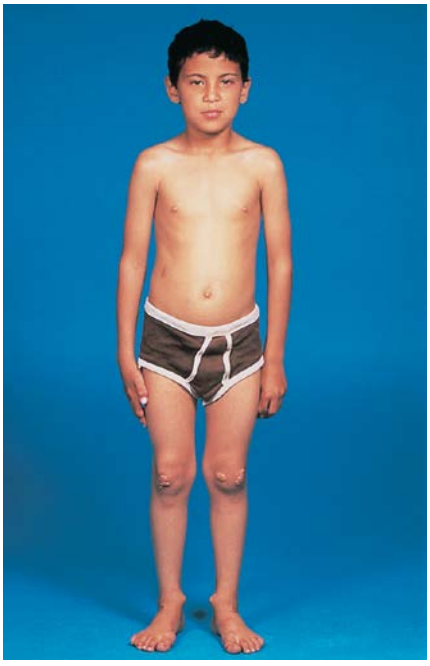

Figure 84.2 MAS: A 12-year-old Egyptian boy with homozygous FH. In addition to multiple xanthomas, he had severe aortic stenosis and died in an attempt at surgical correction. He had a nine-year-old affected brother and the parents were first cousins.

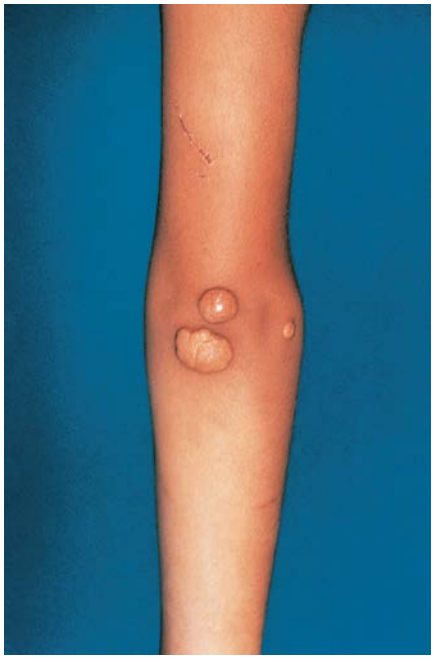

Figure 84.3 MAS: Xanthomas over the elbow.

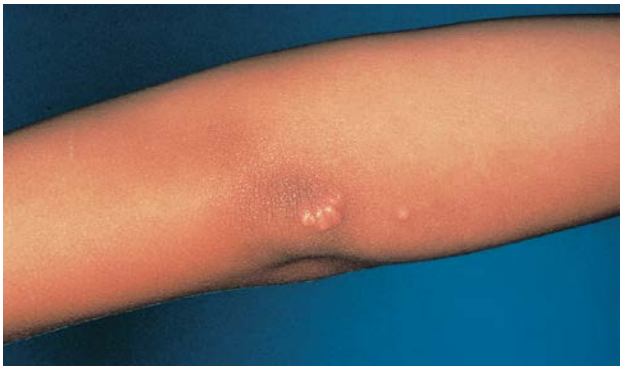

Figure 84.4 MAS: Cutaneous xanthomas over the knee.

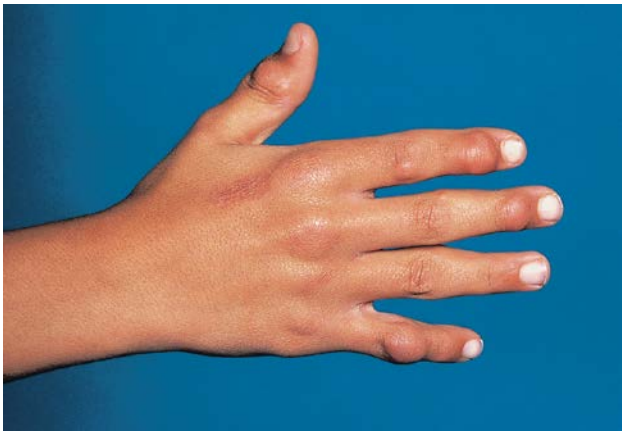

Figure 84.5 MAS: Tendinous xanthomas were evident over each metacarpophalangeal joint, the distal interphalangeal joints of the first, second, and third fingers, and the proximal interphalangeal joints of the second and fifth fingers.

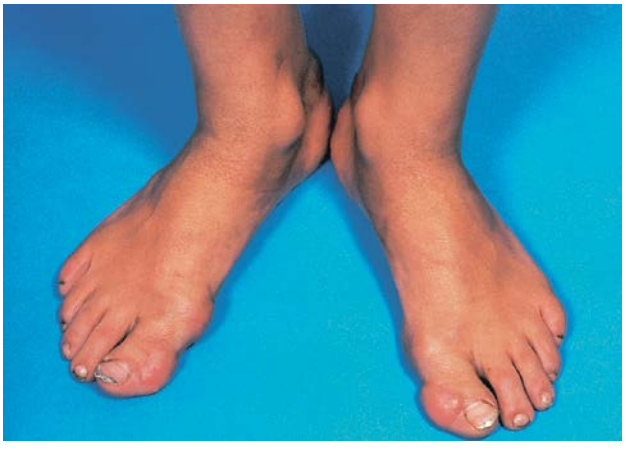

Figure 84.6 MAS: There were medial xanthomas of both feet.

progressive. Patients may have clinical angina as early as five years of age. Myocardial infarctions have been recorded as early as 18 months and three years of age. Most patients have died of this disease by 30 years of age [17].

There is evidence of genetically determined phenotypic variation in that homozygotes who have no LDL receptor function tend to have more relentless disease (with a mean age at death of 11 years) than those with defective receptors with some function (only one of 26 patients had died by the time of the report [18] and he was 23 years old). There is also evidence that other genes or factors modify the expression of the disease. In one family of two siblings

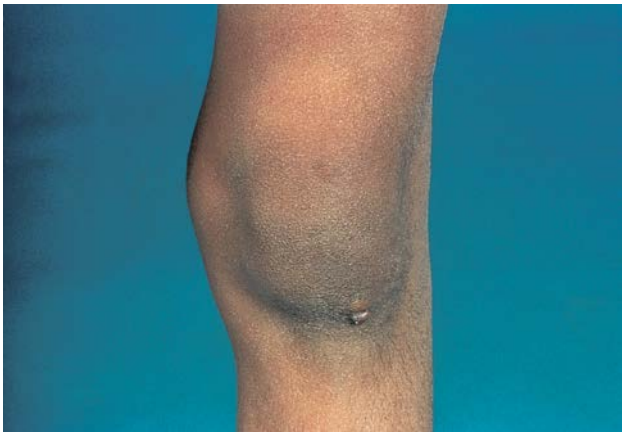

Figure 84.7 BASJ: A seven-year-old with homozygous FH. There were xanthomas over the Achilles tendon and the elbows, as well as the knees, from the age of three years.

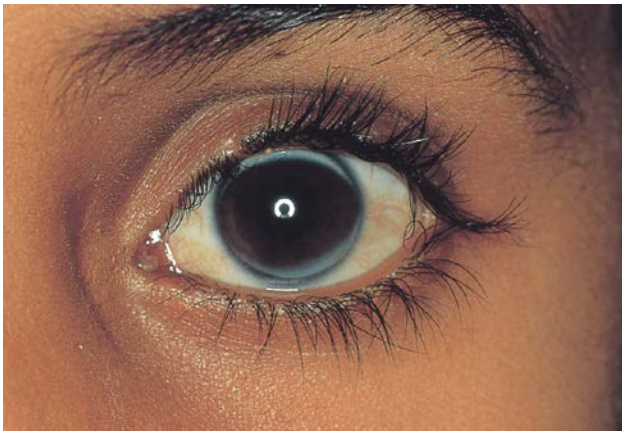

Figure 84.8 BASJ: At seven years, the arcus senilis was well developed.

with the identical mutation in the LDL receptor gene, one died at three years but the other was asymptomatic until 14 years [19]. In an effort to enhance the assessment of risk of vascular disease, a cholesterol-year score has been developed at the National Institutes of Health, similar to the pack-years score for cigarette smokers. By multiplying the number of years a patient has had a certain level of cholesterol (mg/dL), a score is achieved which in a series of 11 consecutive homozygous patients correlated very well with the development of angina [20].

Xanthomatous deposits are also found on the endocardium and on the mitral and aortic valves, leading to regurgitation and stenosis [21, 22]. The aortic valve hemodynamics may be indistinguishable from rheumatic or calcific aortic stenosis [2, 23, 24]. A diagnosis of acute rheumatic fever may also be suspected, on the basis of migratory painful joints and an elevated sedimentation rate, which patients may also have [25, 26]. Recurrent attacks of arthritis or tenosynovitis occur in the ankles, wrists, and proximal interphalangeal joints [22, 26, 27].

These symptoms tend to last for three to 12 days and subside spontaneously. The elevated sedimentation rate may be present in the absence of arthritis, or absent when joint symptoms are present [26]. There may also be a two-fold elevation of the plasma concentration of fibrinogen. Ischemic optic neuropathy that has been referred to as non-arteritic (NAION) may occur before the age of 49 [27].

Prenatal diagnosis has been recommended in the African population [28].

Heterozygous familial hypercholesterolemia

In heterozygotes, concentrations of cholesterol in the blood range from 270 to 550 mg/dL (7–14 μmol/L [29]; mean levels approximate 350 mg/dL (9 μmol/L) [13, 14, 29, 30]. Levels of triglycerides are usually normal. Phospholipids are slightly elevated: they are more consistently elevated in homozygotes [31]. The increased cholesterol content of the plasma is entirely in the LDL fraction [3, 4, 32]. The normal concentration of LDL cholesterol is 110 ± 25 mg/dL. In heterozygotes, the mean levels reported [13] were 241 ± 60, whereas in homozygotes the mean was 625 ± 160 mg/dL. Levels of high-density lipoprotein (HDL) cholesterol tend to be a bit lower in both heterozygotes and homozygotes than in normals. In the absence of hypertriglyceridemia, an elevated level of cholesterol in the blood indicates that LDL cholesterol level is elevated.

Heterozygotes usually develop xanthomas by the time of death [29]. They occur typically over tendons such as the Achilles. Heterozygotes regularly develop xanthelasma, the palpebral xanthoma that is rarely seen in homozygotes. People with normal concentrations of cholesterol may also develop xanthelasma [3]. The corneal arcus is also seen in people with normal lipid metabolism, but in this disease, it occurs at under 45 years of age. In heterozygotes, it is found in 10 percent by 30 years of age and in 50 percent of those over 30 years [32].

Clinical manifestations of coronary artery disease appear in heterozygotes as early as the fourth decade [33]. Its pattern is much more variable than in homozygotes. Mean age at death in males was 55 years and in females 64 years [34]. The probability of a coronary event was 16 percent by the age of 40 years and 52 percent by the age of 60 years in males [35]. In females, the probability was 33 percent by the age of 60 years. Echocardiography may reveal proximal stenosis of the aorta and of the aortic valve. Ultrasonography of the abdomen may be employed to detect calcifications of the abdominal aorta.

GENETICS AND PATHOGENESIS

FH is classically caused by mutations in the gene for the LDLR (Figure 84.1). It is also caused by mutations in the APOB and in the *PCSK9* gene, the latter coding for the proprotein convertase subtilisin/kenin type 9. In 1358

French probands with autosomal dominant FH, mutations in LDLR were found in 1003 patients; of these mutations, 46 percent were missense, 13.6 percent frameshift, 11.3 percent nonsense, and 9.7 percent major rearrangements [36]. Mutations in the APOB gene were found in 6.6 percent of patients and in the PCSK9 in 0.7 percent of patients. Finally, in 19.0 percent of the probands, no mutation was found [36]. Further evidence of genetic heterogeneity in FH was evidenced by a large French family in which mutations could be found in none of the three genes [37]. A genome-wide scan led to the discovery of another disease-causing gene named *HCHOLA4* which was located at chromosome 16q22. It was concluded that there are other FH-related genes because in nine families, mutations were found in none of the four genes.

There are a number of allelic gene mutations in *LDLR*, the gene for the receptor [36–40] in a locus on the short arm of chromosome 19, which was placed at p13.1 to 13.3 [41]. Heterozygotes are found in a frequency of one in 500 [42]. This appears to be the most common single gene disease in humans, and it is seen throughout the world. It is expressed as dominant in heterozygotes. Homozygotes have two abnormal copies of the gene.

Cultured cells require cholesterol for survival. It is a necessary component of the plasma membrane of the cells. Amounts in excess of what is required are stored as cholesterol ester. Mammalian cells grown in serum utilize its LDL rather than synthesizing the compound [43]. The critical component in the uptake of cholesterol from LDL is the highly specific receptor that binds the apoprotein B-100 of the LDL [44–46]. Lipoproteins transport lipids to tissues following hydrolysis catalyzed by lipoprotein lipase (LPL) (Chapter 86); metabolism proceeds through intermediate metabolites, chylomicron remnants and very low-density lipoprotein (VLDL) remnants. Both are rich in cholesterylesters and apoE, and are considered atherogenic lipoproteins, since they accumulate in the arterial walls [46]. An automated method for measuring remnant lipoprotein cholesterol has been shown to be fast and accurate and to be of use in monitoring situations such as postprandial increase in lipids and the metabolic syndrome [46].

LDL is a large, spherical particle with an oily core made up of many cholesterol molecules in ester linkage to fatty acids. There is a hydrophilic phospholipid coat in which one large protein (apoprotein B-100) is embedded. This apoprotein is recognized and bound by the receptor, which is an acidic glycoprotein. The receptor has been solubilized and purified to homogeneity. Its apparent molecular weight is 160 kDa [47–49]. It is synthesized as a precursor with an apparent molecular weight of 120 kDa and, 30 minutes after synthesis, it is converted to the apparently larger form and inserted into the plasma membrane.

LDL is taken up or internalized by a process termed receptor-mediated endocytosis [50–52]. The receptors are found in coated pits of the plasma membrane, where the surface is indented (Figure 84.1). Pits, containing bound LDL, invaginate and pinch off to form coated vesicles, which migrate to lysosomes and fuse with them. There, hydrolysis takes place to yield free cholesterol.

Intracellular cholesterol regulates its own intracellular concentration by means of an elegant system of feedback controls [53]. Some is required in the synthesis of cell surface membranes and in specialized cells, such as the adrenal and liver, and some is converted to steroid hormones and bile acids. Excess may be stored as cholesterol ester, and the enzyme catalyzing this, acylCoA:cholesterol acyltransferase (ACAT), is activated by cholesterol [54]. The rate-limiting step in intracellular cholesterol synthesis is catalyzed by 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase. Regulation occurs at this level in that cholesterol suppresses the synthesis of this enzyme, thus turning off cholesterol biosynthesis [55]. The third regulatory process is a turning off by cholesterol of the synthesis of LDL receptors [56]. This prevents entry of additional LDL and the overloading of cells with cholesterol.

These considerations have relevance to the pathogenesis of atherosclerosis and coronary artery disease in normal individuals who do not have a defect in the LDL receptor. The consumption of a diet rich in dairy products, eggs, and animal meats provides enough cholesterol to overload the system and turn off the synthesis of LDL receptors. This protects the cell against too much cholesterol, but then excessive amounts of LDL accumulate in the blood and cholesterol is laid down in atherosclerotic plaques.

The LDL receptor is a cell-surface glycoprotein containing both N-linked and O-linked oligosaccharide chains [57]. The protein is synthesized in the endoplasmic reticulum and then migrates to the Golgi complex; in the process, the mannose-rich portion of the precursor protein is reduced and O-linked sugars, including sialic acid, are added to the core N-acetylgalactosamine [57]. Next, the receptor moves to the cell surface to cluster in the coated pits, which are lined by a surface protein, clathrin [58]. The coated pits invaginate to form endocytic vesicles, which fuse to form endosomes. The pH in the endosome falls, and LDL dissociates from the receptor which then returns to the surface ready to initiate another cycle of LDL binding and transport.

The gene contains 18 exons spanning 45 kb. The mRNA is 5.3 kb; it codes for a protein of 860 amino acids [59]. The very large number of mutations, so far identified, have fallen into five classes representing the five phenotypic groups [12, 14, 57, 60–69]. Class 1 mutations are null alleles which produce no immunoprecipitable protein. At least one class 1 allele was found in over half of 128 fibroblast lines studied. Among 32 of these alleles studied, 12 had large deletions [12] recognizable on Southern blots. Four French-Canadian homozygotes were found to have a deletion over 10 kb involving the promoter and exon 1 [61]. A different 5 kb deletion of exons 13–15 leads to a truncated mRNA, but no protein [62]. In patients with two null alleles, there is no immunoprecipitable protein and receptors cannot be seen electron microscopically [12, 50].

In class 2 mutations, the proteins synthesized fail to be transported to the Golgi and the receptors accumulate

intracellularly. These, too, are relatively common. Two of them were small deletions in exon 4. Deletion in exon 4 is the mutation for the Watanabe rabbit (WHHL), an animal model for FH [63, 64]. These rabbits have less than 5 percent of the normal number of LDL receptors, and high circulating levels of LDL and cholesterol, and they develop atherosclerosis and infarctions by two years of age. A nonsense mutation in which a single nucleotide substitution produces a stop codon that leads to a truncated protein has been called the Lebanese allele and has been found in several unrelated Arab patients [65]. A majority of class 2 mutants are leaky in the sense that some transport remains.

In class 3 mutations, the proteins are synthesized and they are transported normally, but their structure is altered so that they fail to bind the LDL properly. Levels of binding activity range from 2 to 30 percent of normal. Three mutations documented included two deletions and a duplication; the former do not bind LDL, indicating deletion in the ligand-binding domain, while the product of the duplication binds reduced quantities of LDL [57]. Differential effects on binding are illustrated by FH Paris-1 in which deletion of exon 5 leads to binding of VLDL, but not LDL [61], while FH French Canadian-3 binds neither [70].

Class 4 mutations are altered in their ability to cluster in the coated pits and consequently they fail to internalize bound LDL. These defects are rare, but interesting. Among mutations identified early, there were two deletions, an insertion, a nonsense mutation, and a missense mutation [66]. The stop codon in the nonsense mutation leaves only two of the normal 50 amino acids in the cytoplasmic domain. The insertion adds eight amino acids in this domain and the missense mutation changes a tyrosine to a cysteine at the 80th amino acid of this domain [67]. These observations suggested that this area is critical for binding to a protein as a requirement for movement into the clathrin-coated pits. This was the first evidence that clustering in the pits was required for transport into cells. The two deletions appear to constitute a different subclass of defects in which the membrane spacing domain is altered, and the truncated receptors are largely secreted into the medium of cultured cells [66].

Class 5 mutations code for receptors that cannot discharge the ligand in the endosome and thus cannot recycle to the cell surface. The receptor is then degraded. Deletion of exons 7–14 in FH Osaka-2 leads to this phenotype [71]. A small number of mutations has been found in the promotor [72–74].

Exon-by-exon sequence analysis (EBESA) identified mutations in the *LDLR* gene; p.R3500W was found in eight probands of many Taiwanese families; of them, 25 were missense, five nonsense, and six frameshift mutations in 52; 11 probands were novel. Of the 42 probands with no mutations detected by EBESA, eight had abnormal multiple ligation-dependent probe amplification (MLPA) patterns, with six deletions. Mutations did not correlate well with lipid profiles or failure to lower LDL with statins [75]. A mutation G918>T of the PCSK9 (p.R306S) exon 6 was reported in a Chinese family [76].

Double heterozygosity for mutations in the *LDLR* gene and the *APOB* gene has been reported [3]. A combination of p.Leu479Pro in *LDLR* and p.Arg3527Gln was found in a 15-year-old girl. A double heterozygosity for *LDLR* and *PCSK9* has also been observed [77]. In general, these combinations lead to more severe disease. Two types of mutations should be considered in a family with an LDLR mutation and hypercholesterolemic relatives who do not carry the mutation.

Prenatal diagnosis of homozygous FH has been made [78] by assay of LDL receptor activity in cultured amniocytes. In a series of pregnancies at risk, one was homozygous affected, two were heterozygotes, and one was normal. Prenatal diagnosis of an affected fetus has also been made by fetal blood sampling and analysis of cholesterol [79]. If the mutation is known, molecular diagnosis would be the procedure of choice. Heterozygosity for the Lebanese mutation has been documented in chorionic villus material [80].

Heterozygosity has been diagnosed in cord blood [13], as well as prenatally, but the assay of cord blood is not a reliable method of screening the general public. Even at the age of one year, when the methodology is more accurate, family study would be required to determine that an elevated level of LDL cholesterol is caused by FH. Defective LDL receptor function can be documented in cultured fibroblasts or lymphocytes [79]. DNA-based diagnosis is feasible in populations in which a particular mutation is common. Patients with heterozygous hypercholesterolemia who inherited the gene from their mother had slight but significant increases of total cholesterol, LDL-C, and APOB levels later in life than those who inherited the gene from their father, suggesting maternal programming during pregnancy [81].

Among 1350 patients with FH who were negative for variants in LDLR, APOB, and CSK9, synonymous variants in LDLR, R85R and C186G were identified which affected splice sites [82].

Defective APOB results from a missense mutation (Arg 3500 Gln) in the LDL receptor binding domain. There are other less frequently encountered mutations. Plasma levels of LDL cholesterol are elevated and triglycerides normal.

Patients with autosomal recessive hypercholesterolemia have severe elevations of cholesterol and had large xanthomas on the tendons [84]. Mutations in the *ARH* gene which codes for an adaptor (ARH) protein appears to be involved in the internalization of the LDL-LDC complex.

TREATMENT

Homozygotes

Transplantation of the liver has been performed in a six-year-old child [85] with homozygous disease. The LDL receptors of the transplanted liver removed cholesterol

from the plasma at a near normal rate and effectively reversed the abnormal concentrations. In another patient successfully transplanted, lesions in the coronary arteries regressed, as did xanthomas [20]. Experience with a small number of other patients confirmed dramatic fall in plasma LDL [86].

Homozygotes are generally resistant to the drugs and diet that are effective in heterozygotes, but those with some functional receptor activity may respond. The most practical and effective approach to the treatment of most homozygotes has been the removal of LDL by plasmapheresis or LDL apheresis [20, 87]. These procedures lower blood concentrations of cholesterol appreciably, and xanthomas have been observed to regress, as have lesions in the coronary arteries, which were limiting flow. Currently, this appears to be the treatment of choice [88]. LDL apheresis was found to decrease levels of ferritin, transferrin, and vitamin B12 significantly, and some patients became mildly anemic, but there was no change in plasma iron saturation or folic acid [89].

Lowering of cholesterol levels has also been reported following portacaval anastomosis [90].

Gene therapy has been undertaken in homozygous FH [91]. In this *ex vivo* technique, hepatocytes were isolated from the patient and grown in culture, transfected with the normal gene, and reinjected into the portal circulation. The procedure was effective in lowering cholesterol in the WHHL rabbit and a human protocol was approved, but results were disappointing.

Germ-line interruptions in the *LDLR* and *APOBEC1* genes in mice provide a model for homozygous FH. Introduction of the gene for mouse *LDLR* with an adenovirus vector to the livers of *LDLR*(−/−)*APOBEC1*(−/−) mice led to an 87 percent regression of atherosclerotic lesions [92].

A porcine model of FH has been proposed to test the efficacy of drug eluting stents because it is thought to be a better model of intimal response, similar to that of man [93].

Heterozygotes

Two major approaches have been developed for the treatment of heterozygotes. These patients have one normal LDL receptor gene that is known to be under feedback control. The first group of drugs to be employed is anion-binding resins such as cholestyramine and colestipol.

They prevent the recycling of bile acids and thus stimulate the synthesis of LDL receptors and lower LDL cholesterol concentrations by 15–20 percent. This was enough to reduce the incidence of myocardial infarctions by 20 percent in a ten-year prospective study [38]. In a double-blind study [94] using 8 g of cholestyramine in children, LDL cholesterol was decreased 16.9 percent while it increased 1.4 percent in the controls. Side effects were common, and one boy had intestinal obstruction. This approach is limited by the fact that the cell also responds to cholesterol deprivation by induction of HMG CoA reductase synthesis and by increasing *de novo* synthesis of cholesterol, which inhibits the synthesis of new LDL receptors.

The development of drugs that inhibit cholesterol biosynthesis by inhibiting HMGCoA reductase has provided a systematic approach to treatment by combination with bile acid sequestration. The first of these drugs was mevastatin (Compactin), a compound isolated from Penicillium [95] that has a side chain resembling mevalonic acid. A more potent inhibitor isolated from Aspergillus differs in structure only in the substitution of a methyl for hydrogen group and is called mevinolin or lovastatin [96]. Both compounds lower blood levels of LDL and cholesterol and significantly increase LDL receptors. Combined therapy with cholestyramine and mevinolin lowers LDL cholesterol levels by 50–60 percent [97]. Currently, lovastatin and chemically modified natural statins (pravastatin and simvastatin) or synthetic statins (fluvastatin, cerivastatin, and atorvastatin) are in use in human therapy, not only for FH heterozygotes, but for many others with hypercholesterolemia [98]. These drugs lower LDL cholesterol. They are not without side effects, including hepatic toxicity and myopathy, which may manifest as rhabdomyolysis. The addition of nicotinic acid to the regimen may further improve the effect on levels of cholesterol [97].

In a study in Japan, the age at onset of coronary artery disease was 58.8 ± 12.5 years in 25 patients treated with statins at the onset of the study, which was significantly higher than that of the 76 patients not treated (47.6 ± 10.5 years) ($p < 0.001$). The average age of onset of coronary disease was significantly higher after widespread use of statins in Japan [99]. In heterozygous FH, statin therapy was found to yield a decrease in LDL cholesterol and a slower increase in carotid intimal/medial thickness [100].

In children with FH, diet has been the predominant mode of treatment. Anion exchange resins, such as cholestyramine and colestipol may be effective, but they are unpalatable and poorly tolerated. In a search of statin trials reviewed from Medline, eight randomized, double-blind, controlled studies were found involving 897 patients less than 18 years of age. Therapy with statins was found to reduce serum LDL cholesterol levels by 23–40 percent. Safety was evident in no differences in transaminases or creatine kinase, but long-term safety remains to be determined [101]. Pediatric patients with FH treated with antihyperlipidemic agents have not only decreased levels of serum lipid [75], but also of plasma von Willebrand factor antigen which might further impede the development of the atherosclerosis [102].

In an approach to complications of statin therapy, rosuvastatin administration to children with heterozygous FH was found to cause a significant decrease in peripheral blood mononuclear cell CoQ10 concentrations [103].

A novel compound, ezetimibe, that selectively inhibits cholesterol absorption, when used in addition to statins was found to have an additional LDL cholesterol-lowering effect. There was also a progressive decrease in carotid

intima–media thickness [104]. Reduction in LDL-cholesterol has been about 20 percent [105].

Mipomersen, an oligonucleotide antisense inhibitor directed against apoB mRNA, has been studied in patients with heterozygous FH also receiving conventional lipid-lowering therapy. Significant reduction of LDL cholesterol of 21 and 34 percent were encountered in a dose-related fashion. Levels of APOB were also reduced [106].

All medications which lower lipids are contraindicated during pregnancy [107]. A prudent diet for these patients is one low in cholesterol and saturated fat. Oxidative stress contributes to lipid peroxidation and decreases nitric oxide (NO) bioavailability in atherosclerosis. Long-chain (n-3) polyunsaturated fatty acids (PUFA) are easily oxidized and improve endothelial function. In experimental animals, a fish oil-rich diet increased NO production and endothelial NO synthase expression. A fish oil-rich or supplemented diet appears prudent [108]. Double heterozygotes for mutations in *LDLR* and *APOB* tend to respond to treatment with statins [3]. Monoclonal antibodies to the PCSK9 convertase have emerged as treatment for even homozygous FH [109]. Two injectable human antibodies, Alirocumab and Evolucumab, have been approved by the FDA. They are capable of reducing LDL cholesterol by 50–70 percent.A cholesterylester transfer protein inhibitor Anacetrapib 100 mg daily added to statin therapy reduced LDL cholesterol by 30 percent [110]. Patients with APOB are responsive to statins [83].

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85

Mevalonic aciduria

MAJOR PHENOTYPIC EXPRESSION

Failure to thrive; diarrhea and malabsorption; psychomotor impairment; dysmorphic features, cataracts; retinal dystrophy; hypotonia; hepatosplenomegaly and lymphadenopathy; ataxia; recurrent crises of fever, arthralgia and skin eruption; hyperimmunoglobulin D (IgD); mevalonic aciduria; and defective activity of mevalonic acid kinase.

INTRODUCTION

Mevalonic aciduria was discovered in 1986, the first inborn error in the biosynthesis of cholesterol and nonsterol isoprenoid compounds [1]. It results from a deficiency of the activity of mevalonate kinase (Figures 85.1 and 85.2). The disorder was recognized by organic acid analysis of the urine via gas chromatography/mass spectrometry (GCMS). This compound can easily be missed in GCMS analysis [2]. The development of a stable isotope dilution GCMS assay for Mevalonic acid has facilitated quantification of the compound in body fluids [2].

Mevalonic acid (Figure 85.3) is 3-hydroxy-3-methyl-5-hydroxypentanoic acid. The compound spontaneously cyclizes to form the lactone under the acidic conditions usually employed in liquid partition chromatography, extraction, and other forms of preparation for organic acid analysis. The lactone does not open up under conditions of formation of trimethylsilyl (TMS) derivatives. The

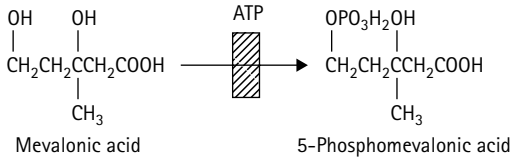

Figure 85.1 Mevalonate kinase, the site of the defect in mevalonic aciduria.

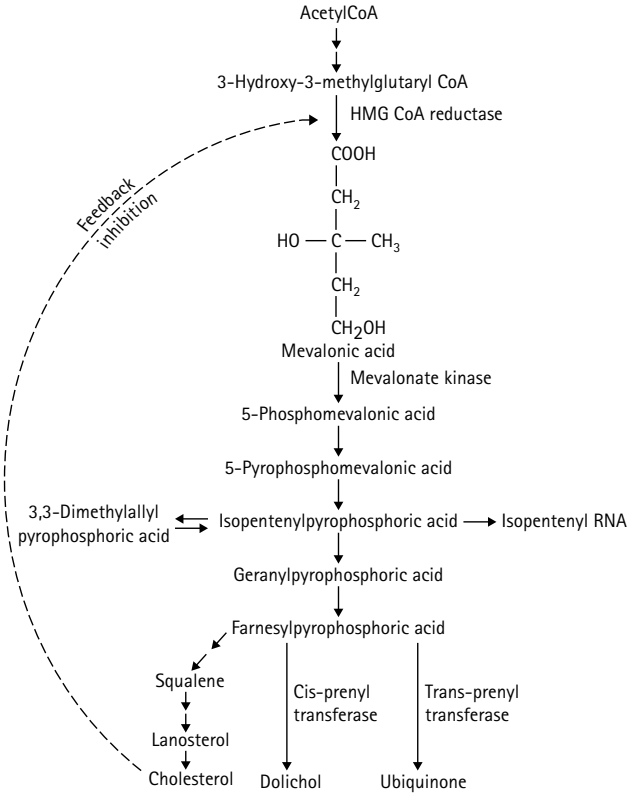

Figure 85.2 Metabolic pathways involving the formation of mevalonic acid and its role in the synthesis of cholesterol, dolichol and ubiquinone.

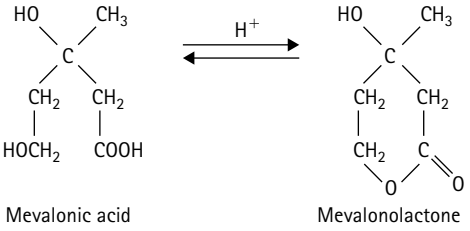

Figure 85.3 Structure of mevalonic acid and its lactone.

mono-TMS derivative of the lactone is formed under acid conditions and the tri-TMS derivative of mevalonic acid is formed after treatment with alkali. Methylation of the acid followed by formation of the TMS derivative provides the best approach to identification.

In the mevalonate kinase reaction (Figures 85.1 and 85.2) the 5-hydroxy group of 3-R-mevalonic acid is phosphorylated to yield mevalonic-5-phosphate. The cDNA for the human enzyme has been cloned and localized to chromosome 12q24 [3]. At least 63 mutations have been defined [3–5], and genotype–phenotype correlations are emerging. Most mutations appear to affect stability or folding of the enzyme rather than its catalytic properties [5].

CLINICAL ABNORMALITIES

Only 20 patients have been reported [6, 7], but varying degrees of clinical severity have been observed, and the spectrum of phenotypes has been enlarged [8–11]. The most severely affected have died in infancy [1, 8] or childhood [9]. Less severely impaired patients have had developmental delay, ataxia, and hypotonia. Most have had recurrent crises of fever, tender lymphadenopathy, increase in liver and spleen size, arthralgia, and a morbilliform eruption. Acute phase reactants, the erythrocyte sedimentation rate, C-reactive protein and leukocytosis, as well as creatine kinase (CK) and transaminases, are elevated. It has recently been recognized that patients with the hyperimmunoglobulin D and periodic fever syndrome (HIDS) have mevalonic aciduria [12, 13]. Mutations in the mevalonate kinase gene have been found in patients with HIDS indicating that HIDS is a milder, allelic form of mevalonic aciduria [14, 15]. An even more attenuated presentation was observed in a five-year-old with cerebellar ataxia and retinal dystrophy and no febrile crises or hyperimmunoglobulin D elevation [7].

Our first patient [1] presented with severe failure to thrive, diarrhea, and hepatosplenomegaly, a picture that led to referral to gastroenterologists. At 19 months, his weight, height, and head circumference were 4–8 SD below the mean for age. He had little or no subcutaneous fat (Figures 85.4 and 85.5). He died at 21 months [6]. Failure to thrive was present in nearly all the early reported patients: it was described as severe in two [1, 16], moderate in five, and mild in two [6]. The two most severely affected had gastrointestinal symptoms suggesting intolerance to cows' milk. Hepatosplenomegaly was notable in five patients.

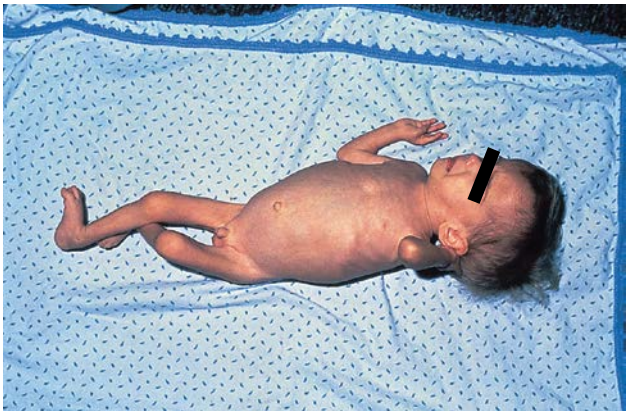

Figure 85.4 ZW: A 22-month-old with mevalonic aciduria. He was tiny and had virtually no subcutaneous fat. The penis was very small.

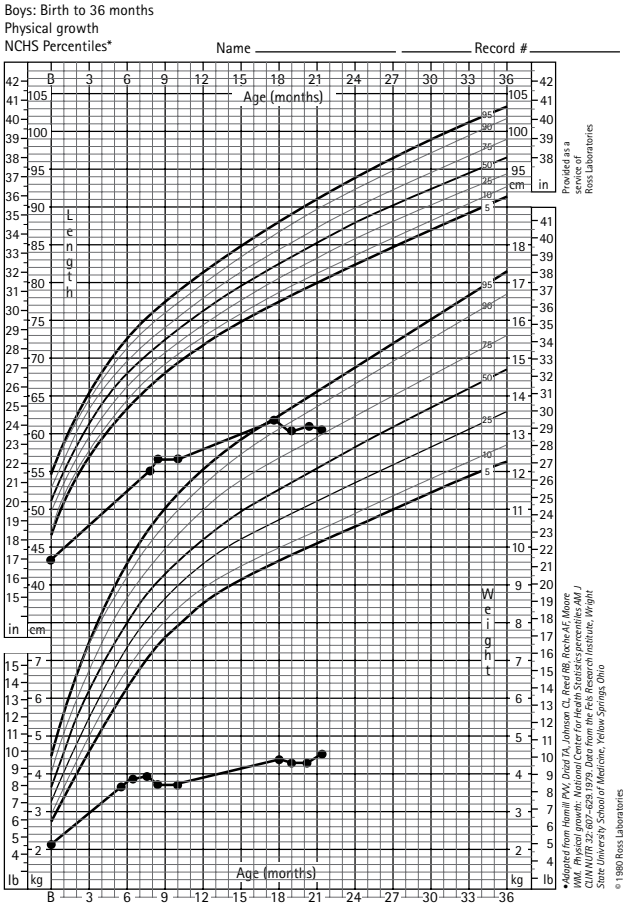

Figure 85.5 Anthropometric data on ZW illustrate the extreme failure to thrive.

Psychomotor impairment has been characteristic of each of the early patients described. It was of such severity in the most severely affected patients that no social interaction was possible [1, 6, 8, 9]. Two siblings [16] had IQs of 60 and 65; in two others [6] IQs were 77 and 82, and in the least severe patient [17, 18] the IQ was 85. Intellectual impairment has appeared to be nonprogressive. In contrast, ataxia

and dysarthria developed after the second year of life in a majority of the patients surviving that long and became progressively more prominent. Imaging of the central nervous system revealed progressive cerebellar atrophy [7]. Deep tendon reflexes may be accentuated, and a crossed response may be elicited. There may be cortical thumbs and incomplete extension at the elbows and knees.

Hypotonia is observed regularly, and some patients have appeared to have myopathy. In one of the least severely affected patients [17], the complete picture was of static myopathy, borderline impaired mental development, and severe ataxia in a 12-year-old. Myopathy became more severe during febrile crises. One patient developed cardiomyopathy and heart block and required artificial ventilation for two weeks. Two patients had febrile convulsions at one and two years of age.

Cataracts were observed in a number of patients [11, 16]. Two others [6] developed uveitis and retinitis pigmentosa, which became worse with crises. Retinal dystrophy may take the form of bone-spicule retinitis pigmentosa or may be more subtle, thinned vessels and uneven retinal surface and abnormal electroretinogram, and there may be optic atrophy [7].

Dysmorphic features were described in all but a few patients [7, 17], but were described as subtle in four [6]. The characteristic picture (Figures 85.6 and 85.7) is of dolichocephaly with frontal bossing, posteriorly rotated low-set ears, antimongoloid slanting of the eyes, a small mouth and jaw, and thin lips. One patient [1] had a small penis and a congenital hydrocele. In this boy, closure of fontanelles and sutures was delayed; by 19 months they were all widely patent. A third fontanelle may be present. Another of our patients had even more delayed closure of sutures, but she was found to have cleidocranial dysplasia and apparently independent mutations in the *RUNX2* gene (Figure 85.8).

Malabsorption was documented by an increase in quantified stool fat in two patients studied [6]. Biopsies of

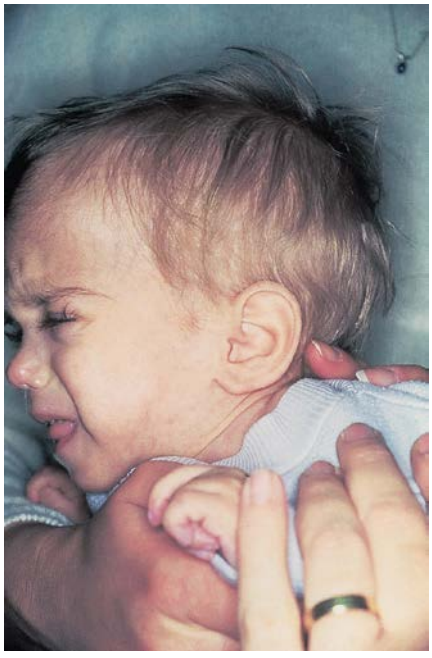

Figure 85.7 Profile illustrates the dolichocephaly and mild hypognathia. This ear was larger than the other.

Figure 85.6 Close-up of the face revealed the prominent forehead and the low-set ears, as well as the long philtrum and thin lips.

Figure 85.8 EF: An infant with mevalonic aciduria. The forehead was long, and a V-shaped open fontanel was visible. She was heterozygous for two mutations, p.P263R and p.I268T, performed by Dr HR Waterham of the University of Amsterdam, the first of which was novel. Mevalonate kinase activity was 0, performed by Dr Michael Gibson then of the University of Oregon. In addition, she had cleidocranial dysplasia and a heterozygous mutation in the *RUNX2* gene, p.Y394X, a novel mutation leading to a truncated protein reported by Dr Christine Eng at Baylor University.

the duodenum revealed no abnormalities. Bile acids were found to be in normal concentration in serum, urine, and feces [6] and studies of the pool size, fractional turnover rates, and rates of synthesis of bile acids appeared to be normal [19].

Electroencephalograms were carried out in eight patients, five of which were normal, and three showed generalized slowing. Neuroimaging was most striking for the presence of cerebellar atrophy [6, 7]. In one patient, a normal magnetic resonance image (MRI) at 18 months when he was presymptomatic was followed over the next three years by the parallel development of severe ataxia and cerebellar atrophy. In some patients, there was also some cortical atrophy. Neuropathologic examination in one patient confirmed loss of cerebellar mass, including the entire vermis. Histology of the muscle was consistent with atrophy. Myopathy or hypotonia may lead to kyphoscoliosis.

The recurrent crises of fever have been associated with vomiting and diarrhea. Careful search has failed to reveal infectious agents. They occurred up to 25 times per year and averaged 4–5 days in duration. Four patients died during crises. Some have had arthralgia, subcutaneous edema, and a cutaneous eruption during the crisis.

Most patients with HIDS exhibit only the recurrent febrile crises and do not have neurologic abnormalities or dysmorphic features [20]. On the other hand, elevated levels of IgD have been found in 100 percent of patients with the classic mevalonic aciduria phenotype [7], and they rise during acute febrile crises [13]; as do the acute phase reactants. Elevated IgD may be absent in a patient with neurologic features and retinal dystrophy and an absence of periodic fevers [7].

Two patients have been described [21] in which a severe prenatal or neonatal onset were associated with difficult to detect levels of mevalonic acid in the urine. One presented with fetal ascites and oligohydramnios. There were no dysmorphic features. He died at eight weeks. The other had neonatal hepatomegaly, direct hyperbilirubinemia, and elevated transaminases. He developed severe failure to thrive, hepatosplenomegaly, hugely enlarged lymph nodes, cardiomegaly, developmental delay, a morbilliform rash, and swollen joints. Because of the clinical phenotype mevalonic aciduria was suspected despite absence of mevalonic acid on organic acid analysis of the urine. The diagnosis was made by stable isotope dilution detection of mevalonic acid in the urine of 1220 mg/g creatinine. In the first patients, mevalonic aciduria was also absent on organic analysis; stable isotope dilution revealed 276 μg/mg creatinine. Assay for mevalonic kinase activity in cultured fibroblasts yielded no activity.

Anemia may be sufficiently severe that a number of blood transfusions are required [8]. Some degree of anemia was present in five of the nine patients on whom this information was available. The serum cholesterol concentration may be normal or slightly reduced. Abnormal levels were reported in four patients, but two of these also had normal levels on occasion [6]. The creatinine kinase levels in plasma were markedly elevated in the majority of patients. Values as high as 3000 and 7520 IU/L have been recorded. The highest levels were in the course of acute crises, but the peak elevation followed the peak of symptoms by 2–4 days. The level of CK was positively correlated with the urinary excretion of mevalonic acid. Transaminases (AST and ALT) were also elevated in the majority of patients. Metabolic acidosis is not a feature of this disease.

GENETICS AND PATHOGENESIS

The disorder is autosomal recessive in nature. Two affected offspring were observed in four families, and the sex distribution has been equal [6]. Furthermore, activity of mevalonate kinase intermediate between patient and control was found in lymphocytes freshly isolated from both the father and mother of patients [1, 2]. Prenatal diagnosis of an affected fetus was carried out in a subsequent pregnancy of the index patient by analyzing the amniotic fluid for mevalonic acid. The pregnancy was terminated, and the diagnosis confirmed by assay of the enzyme in fetal tissues.

The molecular defect in mevalonic aciduria is in the enzyme mevalonate kinase (Figure 85.1). In the usual assay, ^{14}C-labeled mevalonic acid and adenosine triphosphate (ATP) are converted to labeled mevalonate phosphate and pyrophosphate [1, 2, 22, 23]. Control fibroblast lysates displayed a mean activity of 1380 pmol/min/mg protein. Lysates of fibroblasts derived from the patient had activities of 0–4 percent of the control mean. In lymphoblasts, activity in the patients was 1–2 percent of the control mean. The enzyme is also active in freshly isolated lymphocytes and defective activity has been documented in lymphocytes of patients [1]. The level of residual activity has correlated poorly with clinical phenotype; the mildest phenotype reported had 0.4 percent of control activity in fibroblasts [7]. In nine heterozygotes, mean activity in lymphoblasts was 47 percent and in seven, activity in fibroblasts was 67 percent of the control mean.

The localization of the gene was more finely defined to 12q24 and narrowed to a 9 cm region [15]. A missense mutation was identified in the index patient with mevalonic aciduria [3], an A-to-C change at nucleotide 902 causing a change of asparagine 301 to threonine (N301T). He was a compound of this mutation and another considered to have been inherited from his mother. Two patients in another family with a relatively mild phenotype were homozygous for a G-to-A change at 1000 yielding a change from alanine 334 to threonine [4, 7]. Another patient was homozygous for I268T [24]. The patient with the mildest phenotype [7] was a compound of A334T and 72insT [7]. In patients with HIDS, four missense mutations and a 92 bp deletion were identified [14, 15]. One mutation (V337I) was found in nearly all the patients studied, usually compound. Enzyme activity was reduced in all, but not completely deficient. Four novel mutations were found [25] in a cluster: L243I, L264F, L265P, and L268T, the last found in a Mennonite

family. Bacterial expression assay confirmed the enzyme-deficient nature of these mutations.

Overall, the majority of mutations have been missense [5]. The mutation p.V377I continues to be relatively frequently encountered and exclusively associated with the HIDS (hyperimmunoglobulin D periodic fever syndrome), usually as one of two disease genes. Under *in vitro* conditions designed to improve protein folding in cultured fibroblasts, the phenotype of residual mevalonate kinase activity correlated well with that of enzyme protein indicated by immune blotting. Mutations p.V377I, p. A148T, p. N205D, and p.T209A, along with temperature sensitivity, are concerned primarily with folding or stability of the enzyme. Patients with the classic mevalonic kinase phenotype tend to have very low residual activity in this assay, but even some of these exhibited culture conditions dependent increase in activity. The p.A334T mutation does appear to be a Km mutant, affecting the binding of substrate [5].

At least 79 mutations have been reported [21]. Mutations p.I268T/R388X and homozygosity for each of these mutations have been found in more than one patient with severe disease.

In the presence of the metabolic block, mevalonic acid accumulates in body fluids. Quantitative analysis of the urine of ten patients revealed a massive excretion ranging from 900 to 56,000 mmol/mol creatinine, while normal subjects excrete a mean of 0.16 mmol/mol creatinine. Excretions of 900–1700 mmol/mol creatinine were found in patients with milder disease. Plasma concentrations in patients have ranged from 30 to 540 mmol/L; the control mean was 0.026 mmol/L. Mevalonic acid clearance by the kidney is very efficient and it appears to involve active renal tubular secretion. A patient with a low level of mevalonic aciduria (51–69 mmol/mol creatinine) was found to have a relatively large amount of residual activity of the enzyme [22].

The pathogenesis of the clinical manifestations of mevalonic aciduria is not clear. Mevalonic acid occupies a unique place in intermediary metabolism. It is an important precursor in the biosynthesis of cholesterol and other sterols, dolichol, and ubiquinone, as well as nonsterol isoprenes involved in the formation of membranes, the glycosylation of proteins, the respiratory chain, and the replication of DNA [26, 27] (Figure 85.9). The enzyme is present in peroxisomes as well as the cytosol.

The pathway is regulated via feedback inhibition by cholesterol of the synthesis of mevalonic acid at the 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase step. When cholesterol, which is ingested or derived from plasma low-density lipoproteins (LDL), downregulates HMG CoA reductase, nonsterol isoprenoid synthesis is preserved by inhibition of squalene synthetase and more distal enzymes, further limiting the incorporation of farnesylpyrophosphate into cholesterol. The initial enzymes in the nonsterol branches of the pathway have a very high affinity for farnesylpyrophosphate [26, 27].

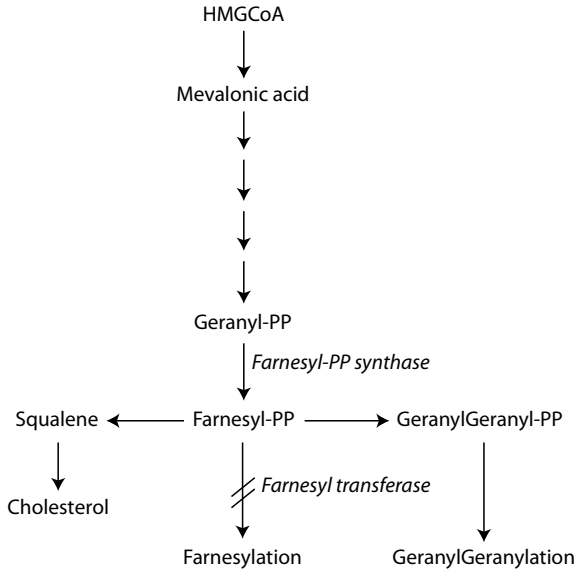

Figure 85.9 Action of farnesyltransferase inhibitors on the mevalonate pathway of cholesterol biosynthesis. Abbreviations include: HMGCoA, hydroxymethylglutarylCoA; PP, pyrophosphate; ManA, manumycin A; Tip, Tipifarnib; Lon, Lonafarnib.

Provision of exogenous cholesterol was without evident effect on patients, indicating (along with their relatively normal levels of cholesterol) that the clinical disease is not a consequence of a shortage of cholesterol. On the other hand, a direct test of the hypothesis that mevalonic acid itself was toxic, by inhibition of HMG CoA reductase with lovastatin, resulted in a severe clinical crisis with rhabdomyolysis [6]. These observations have focused on the possibility of diminished synthesis of a nonsterol isoprenoid product of the pathway.

Ubiquinone concentrations in plasma were found to be reduced in four of six patients studied. Levels were consistently below the control range, though not very far below. Ubiquinone is important for cardiac and muscular function. Concentrations of leukotriene E4 in the urine of patients were found to be highly elevated [6, 28]. Furthermore, in the two patients given lovastatin there was a further 20 percent reduction in ubiquinone.

The recognition that this defect causes HIDS could provide clues as to the pathogenesis of these diseases. Studies of 2^{14}C-mevalonate in rats indicated a difference in metabolism in the and skin where there was formation of labeled fatty acids palmitate and stearate, through a postulated shunt mechanism, from the liver where there was no labeling of fatty acids [29]. It has become clear that the elevated levels of leukotrienes and IgD are secondary [7]. In addition, the febrile crises appear to diminish with increasing age.

The observation that mutant mevalonic kinase activity can be increased in cultured fibroblasts by a chemical chaperone approach to improving protein folding [5] raises the possibility of therapeutic approaches to improving activity with small molecules.

TREATMENT

Trials of supplemental cholesterol, bile acids, and inhibitors of HMG CoA reductase have not been therapeutic. Corticosteroid therapy appears to ameliorate acute crises [6]. Supplementation with ubiquinone may be of interest.

Some success has been reported in treating febrile episodes with anakinra (1.5 mg/kg/day), the interleukin-1-receptor antagonist [30].

The systemic inflammatory disease that characterizes mevalonic aciduria has been related to the secretion of caspase-1-dependent IL-1β, and this has been related to a shortage of geranylgeranylpyrophosphate (GGPP). This has led to the use of farnesyltransferase inhibitors tipifarnib and lonafarnib, which had been developed as cancer chemotherapeutic agents [31, 32]. Success in treating murine monocytic cell lines *in vitro* was followed by their use in monocytes from two patients. Lipopolysaccharide induced secretion of IL-1β was significantly reduced. The mechanism was thought to involve recovery of GGPP flux. Allogeneic bone marrow transplantation has been reported [31] in a three-year-old who had sustained remission from febrile attacks and inflammation. Cord blood stem cell transplantation in a two-year-old yielded sustained remission from Febrile attacks and inflammation [33], and neurologic and psychomotor development were normal after years at the time of report.

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86

Lipoprotein lipase deficiency/type I hyperlipoproteinemia

MAJOR PHENOTYPIC EXPRESSION

Creamy appearance of fasting plasma, episodic abdominal pain, pancreatitis, eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, increased concentration of triglycerides in plasma, hyperchylomicronemia, type I hyperlipoproteinemia and deficient activity of lipoprotein lipase.

INTRODUCTION

Some of the clinical characteristics of familial hyperchylomicronemia were recognized as early as 1932 in the report by Burger and Grutz [1] of an 11-year-old boy, the product of a first-cousin mating, in whom cutaneous xanthomas and hepatosplenomegaly were associated with creamy fasting plasma. Holt and colleagues [2] in 1939 reported two siblings with gross hyperlipemia. The proband had severe attacks of abdominal pain. The defect in lipoprotein lipase in postheparin plasma was defined by Havel and Gordon [3] in 1960 in a study of three siblings with fasting hyperchylomicronemia. The initial patient of Holt *et al.* [2] was followed by Knittle and Ahrens [4] and shown by Frederickson and colleagues [5] to have defective activity of postheparin lipoprotein lipase (EC 3.1.1.3) activity. The enzyme requires a cofactor, apolipoprotein CII, and catalyzes the hydrolysis of the triglycerides of chylomicrons. Clearance of chylomicrons from plasma is impaired leading to accumulation of triglycerides. Deficiency of the apoenzyme or the cofactor can cause clinical lipoprotein lipase deficiency. The gene (LPL) for lipoprotein lipase has been mapped to chromosome 8p22 [6], and it contains 10 exons over 30 kb [7]. Over 100 different mutations have been identified, the majority of them point mutations [8].

CLINICAL ABNORMALITIES

The earliest recognition of this disorder is often fortuitous. The lactescence of blood, plasma, or serum (Figure 86.1) is observed in a routine sample drawn for some other purpose. In fact, the obtaining of blood samples is so common in developed countries that it is surprising most patients are not recognized in this way. The disorder may be evident as early as two days (Figures 86.2–86.5), eight days [9], and one month [10].

At the other extreme, asymptomatic patients have been discovered in the course of screening family members. The blood looks like cream of tomato soup. The characteristic appearance of an excess of chylomicrons in the blood is demonstrated by permitting the plasma to stand for 18–24 hours at 4°C. The chylomicrons appear as a creamy layer on top and the infranatant layer is clear. In types III, IV, and V hyperlipoproteinemia both layers are turbid, while in type II the plasma is clear throughout. A supernatant creamy layer is also seen in type V (Table 86.1). The convention for these observations and for quantitative studies of lipid concentrations is that blood samples are obtained after a 12–14-hour fast. In addition, the individual should not have gained or lost weight unusually for two weeks previously, received an unusual diet, or taken any drugs known to affect lipid concentrations. The period of fasting is usually

Figure 86.1 Whole blood of an untreated patient with lipoprotein lipase deficiency. The sample initially appeared uniformly creamy. With standing for 18 hours or more at 4°C, plasma of these patients displays a layer of cream above and a clear layer below.

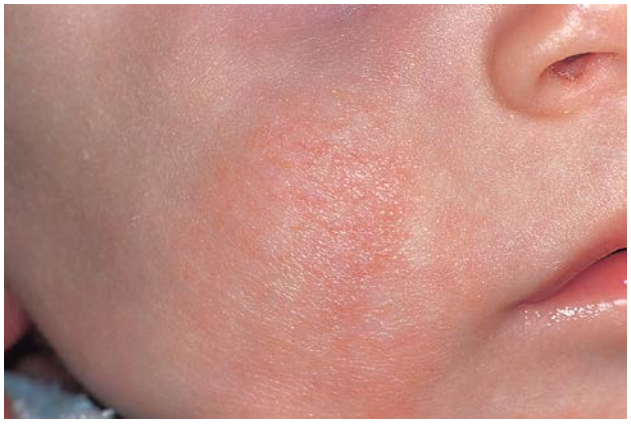

Figure 86.2 JL: A 21-month-old infant with type I hyperlipoproteinemia. The face at time of admission revealed a roughened patch on the cheek containing tiny, pearly xanthomata. These disappeared promptly after dietary lowering of triglyceride concentrations. The patient was admitted because of abdomen distension and found to have a hepatic mass, which was removed and was a benign hemangioendothelioma.

modified in young infants, although our infant withstood a 24-hour fast without hypoglycemia or other complication.

The most common clinical presentation is with acute, recurrent episodes of abdominal pain [11–13]. The age at which this symptomatology begins is quite variable, but it ultimately occurs in virtually all individuals with this disorder. It may occur first in infancy, somewhat later in mid-childhood [12], or not until 20 [10] or 25 years of age. Pains may vary from mild apparently infantile colic to severe peritonitis. They may be quite disabling. They are often generalized or mid-epigastric, but may be localized,

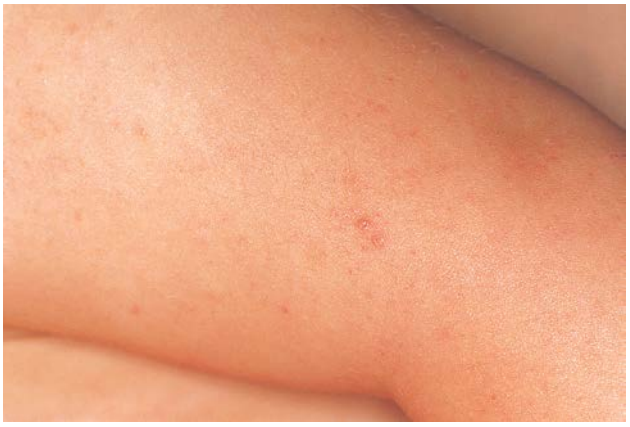

Figure 86.3 JL: Two xanthomata that appeared on the leg after poor compliance with diet.

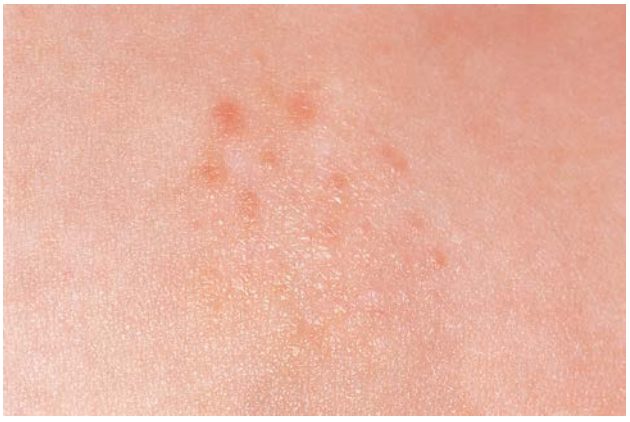

Figure 86.4 A group of xanthomata on the skin of the same patient.

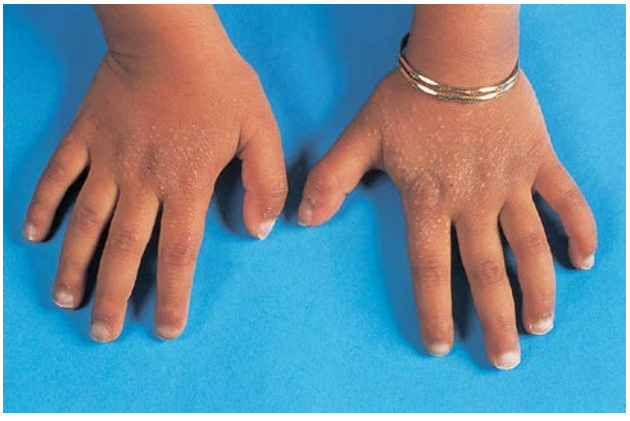

Figure 86.5 Small but plentiful xanthomata characterize lipoprotein lipase deficiency in this four-year-old patient with advanced cutaneous disease. Triglycerides ranged from 2000 to 4000 mg/dL.

Table 86.1 Lipoprotein patterns characteristic of inherited hyperlipidemias

		Lipoprotein patterns					Lipid concentrations		
Type	Current designation	Chylomicroms	VLDL	LDL	HDL	Appearance of plasma[a]	Cholestrol	Triglycerides	Chol/Tri ratio
I	Hyperchylomicronemia	Increase	N or ↑	N or ↓	N or ↓	Creamy on top clear below	N or Sl. ↑	↑	<0.2
II	Familial hypercholesterolemia	Absent	N(IIa) ↑(IIb)	↑	N	Clear, or Sl. turbid	↑	N	>1.5
III	'Broad or floating beta' disease	Absent	β-VLDL[b]	Abn.[b]	N	Turbid, or faint layer of cream	↑	↑	Variable May = 1
IV	Familial hyper-pre-β-lipoproteinemia	Absent	↑	N	N	Turbid, no layer of cream	↑ or N		Variable
V	Familial hyper-pre-β-lipoproteinemia and hyperchylomicronemia	Increased	↑	N	N	Creamy on top turbid below	↑		0.15—0.6

Abbreviations: Abn, abnormal; HDL, high density lipoprotein; LDL, low density lipoprotein; N, normal; Sl., slightly; VLDL, very low density lipoprotein.
[a] After standing for 18—48 hours at 4°C.
[b] 'Floating' β-VLDLI: LDL of abnormal lipid composition.

especially to the right or left upper quadrant. Narcotic dependence has been observed. Pain may be associated with spasm, rigidity, or rebound tenderness. There may also be fever and leukocytosis, and this presentation has led to surgical intervention. Usually, a milky exudate in the peritoneal cavity is the only finding. The viscera may appear pale or fatty. Pains may be accompanied by anorexia and abdominal distension, or by vomiting, or diarrhea.

Acute attacks are always associated with hyperlipemia. In a patient being successfully managed, they may follow dietary indiscretion or noncompliance. They are especially likely to follow the resumption of a normal diet in an individual who had reduced triglyceride levels by dietary restriction. They may also follow intercurrent infection, and attacks have been related to alcohol. Pregnancy may severely exacerbate symptoms [10] and so may oral contraceptive agents. Many patients learn to regulate their dietary intake of fat in a manner sufficient to eliminate the occurrence of abdominal pains [12].

Acute pancreatitis is a well-recognized complication of hyperchylomicronemia [10, 14, 15]. This may cause severe abdominal pain radiating to the back or shoulders and prostration, hypotension, sweating, and shock. It may lead to complete pancreatic necrosis, but does not lead to calcification. Pancreatitis may be fatal [10]. Necrotizing pancreatitis may be recognized at surgery [14], and pancreatic pseudocysts may be found [10], as well as extensive mesenteric fat necrosis and multiple adhesions. The serum amylase level may be very high. On the other hand, the diagnosis of pancreatitis in these patients may be complicated by the fact that the turbid plasma of the patient with hyperchylomicronemia may interfere with the determination of amylase activity in the serum [16, 17]. Normal amylase values have been observed in patients documented at laparotomy to have pancreatitis [18].

These problems may be overcome by serial dilution of the serum if the lactescence is recognized and a true elevation demonstrated [16], or probably better by examination of amylase in the urine, especially the amylase/creatinine clearance ratio [17]. Pancreatitis has been clearly related to the presence of hyperlipemia [2, 14], and dietary reduction of serum triglyceride levels is successful in preventing further attacks. In fact, attacks of pancreatitis appear to occur only when serum concentrations of triglycerides exceed 1000 mg/dL [15], and morbidity and mortality are rare when levels are under 2000 mg/dL [18]. The association between hyperchylomicronemia and pancreatitis is so close that patients with diagnosed pancreatitis or recurrent abdominal pain should be screened for hyperlipemia. In 45 patients with acute pancreatitis examined prospectively, ten were found to have hyperlipoproteinemia [15]. Certainly, infants and children with pancreatitis and patients with familial pancreatitis should be examined for hyperchylomicronemia. In a 31-year clinical follow up of a patient who had 22 episodes of recurrent pancreatitis, he had nevertheless preserved pancreatic endocrine function, no pseudocysts, and no pancreatic calcification [19].

Hepatosplenomegaly is common in type I hyperlipoproteinemia [20]. It may be particularly prominent in infants and children. It is clearly related to fat intake, and the size of these organs can decrease within 24–48 hours of the initiation of the fat-free diet. Generally, some enlargement remains even with long-term dietary management. Occasionally, pains have been

related to the spleen, and the spleen may be quite hard. It also recedes in size with reduction in the intake of fat. Fat embolism may occur in hyperlipemic individuals, and a variety of complications such as seizures, transient paralysis, or gastrointestinal hemorrhage have been attributed to such aggregations of chylomicrons. In one patient, what appeared to be splenic infarcts were seen on angiography, but at surgery the patient had pancreatitis and the removed spleen contained foam cells, but no infarcts [21]. Foam cells have been observed on needle biopsy of the liver [14], representing storage of lipid in macrophages and Kupffer cells.

Among early manifestations in 14 infants with the onset of symptoms prior to one year of age were irritability in seven, lower intestinal bleeding in two, splenomegaly in one, pallor or anemia in four [22]. In this series, one additional patient came to light because of a positive family history, and another was discovered fortuitously. The intestinal bleeding stopped with institution of a low-fat diet. Each patient had lactescent plasma at presentation.

Cutaneous eruptive xanthomas (Figures 86.3, 86.4 and 86.5) have been observed in about 50 percent of patients with type I hyperlipoproteinemia [20]. They cluster preferentially over the buttocks, proximal portions of the extremities and extensor surfaces, but they may occur anywhere, including the skin of the face. Lesions have been seen on the mucous membranes, including the hard palate and tonsils or fauces. They appear as nodules 1–5 mm in diameter. They result from phagocytosis of chylomicroans by macrophages [23]. They may be yellow or have a yellow center, but they may not. They may be erythematous; they tend to be flat. They may coalesce to form larger plaques. However, patients with this disease do not develop tendinous, tuberous, or planar xanthomas, or xanthelasma. The lesions are usually neither painful nor pruritic. They may occur within days of the elevation of plasma triglyceride levels over 2000 mg/dL and have been described as early as the first weeks of life [13]. They may fade rapidly on dietary reduction of these levels, but complete disappearance may take as long as three weeks.

Lipemia retinalis (Figure 86.6) is seen occasionally, but is characteristic of long-standing hyperlipemia. The entire fundus may have a pale or salmon cast, and there may be an increased light reflex over the vessels. The arteries and veins may appear milky-white. There may rarely be white deposits of lipid in the retina; and disturbances of circulation such as microaneurysms and hemorrhages have been reported [24, 25].

It is of interest that patients with type I hyperlipoproteinemia do not appear to be at risk for premature atherosclerotic disease. The numbers of autopsied patients have been small, but none have had appreciable atherosclerotic change at ages ranging from 24 to 42 years of age [10]. Certainly, there has been no clinical evidence of coronary artery disease or cerebral vascular disease [19].

Some patients have been anemic [22], and one patient has been reported with persistent thrombocytopenia, leukopenia, and occasional anemia [26]. Bruising has been reported in another [27]. Slight to moderate hemolysis may be relatively common [28]. One patient [2] had chronic leg ulcers. A group of five patients has been reported with an unusual problem of intermittent swelling of the scrotum, and swelling, along with blueness or mottling, of the legs [12]. Surgical exploration of the scrotum revealed a milky effusion in the tunica vaginalis.

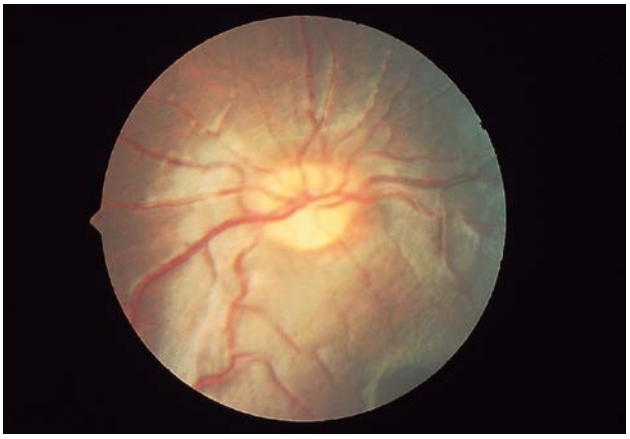

Figure 86.6 Lipemia retinalis in a patient with lipoprotein lipase deficiency.

These patients, unlike those with many forms of hypertriglyceridemia, do not have abnormal glucose tolerance curves, and they do not have hyperuricemia. Secondary diabetes or pancreatic exocrine insufficiency may develop after many attacks of pancreatitis.

The very high plasma lipid may produce artifactual lowering of the values of many plasma solutes, determined in the routine clinical chemistry laboratory. The degree of error is approximately 1 percent for each 0.9 g triglyceride/dL [29]. Thus, in a patient with triglyceride of 10,000 mg/dL, an 11 percent reduction would yield a sodium concentration value of 129 mEq/L for a true sodium concentration of 145 mEq/L in fat-free plasma water. The importance of recognizing this issue is that such patients should not be treated for hyponatremia. On the other hand, lipemia may spuriously elevate levels of hemoglobin and bilirubin [30].

GENETICS AND PATHOGENESIS

Lipoprotein lipase deficiency is autosomal recessive in inheritance. Occurrence in a number of siblings has been reported [2], as has consanguinity [9]. Lipoprotein lipase activity of about 50 percent of normal has been reported in adipose tissue of parents of patients with deficiency [31]. Low levels of a lipolytic activity have also been observed in postheparin plasma of relatives, but heterozygosity cannot always be demonstrated by assay of the plasma [13]. Heterozygotes may have hypertriglyceridemia [31], but fasting levels of triglycerides are usually normal. In fact, it has been demonstrated by careful study of an extended

pedigree [13] that hypertriglyceridemia of many genetic and other causes is so common in adults that the finding of an elevated concentration of triglycerides in a parent or relative cannot be equated with heterozygosity for lipoprotein lipase deficiency.

Analysis of the lipids of the plasma in patients reveals markedly elevated concentrations of triglycerides. In the untreated patient, levels usually range from 1000 to 4000 mg/dL but may be as high as 15,000 mg/dL [29]. Triglycerides constitute 80–95 percent of chylomicrons. Concentrations of cholesterol are normal or moderately elevated. It is only when the triglycerides are very high that the cholesterol rises; the ratio of the cholesterol to triglyceride is always less than 0.2 in type I hyperlipoproteinemia, and often less than 0.1.

Lipoprotein electrophoresis yields a characteristic chylomicron band at the origin. The type I pattern can be demonstrated by electrophoresis or ultracentrifugation as consisting exclusively, or nearly so, of chylomicrons (Table 86.1). The very low-density lipoproteins (VLDL) are normal or slightly increased and the LDL and HDL are usually depressed. Treatment with a low-fat, high-carbohydrate diet usually leads, as chylomicrons fall, to an increase toward normal of LDL and increased levels of VLDL, but those of HDL remain low. The diagnosis of type I hyperlipoproteinemia is often confirmed by the elimination of fat from the diet, after which the chylomicrons disappear from the blood within a few days and triglyceride concentrations fall to 200–400 mg/dL. Most pediatric patients with hyperchylomicronemia have type I hyperlipoproteinemia. Most patients with type V are adults. However, childhood type V hyperlipoproteinemia has been reported [32], and patients with classic lipoprotein lipase deficiency sometimes have a type V pattern with time. Incubation of plasma in 3 percent polyvinylpyrrolidone will separate chylomicrons from other lipoproteins and is thus useful for the diagnosis of hyperchylomicronemia.

The definitive diagnosis of the classic type I disease requires demonstration of the molecular defect in the activity of the enzyme lipoprotein lipase [33–35]. This enzyme catalyzes the hydrolysis of glycerolester bonds in circulating triglycerides at the vascular endothelial surface in tissues, especially adipose. The enzyme is released by the intravenous administration of 60–100 units/kg heparin and is assayed in plasma obtained 10–15 minutes later. The enzyme requires the specific plasma cofactor, apoprotein C-II. It is inhibited by protamine. Concentrations of enzyme in patients are usually less than 10 percent of control levels and may approximate zero. Heparin was originally observed [36, 37] to clear postprandial lipemia in dogs. *In vitro* addition to plasma does not reproduce this effect; injection *in vivo* releases the lipase.

Defective activity of the enzyme may be documented in postheparin plasma or in adipose tissue. Heparin also releases hepatic lipase into plasma. This enzyme has little activity against chylomicrons, and so it created no problem in the original assay of Havel and Gordon [3], but most modern assays are done with artificial emulsions of triglycerides. Selective assay requires inhibition of the lipoprotein lipase with protamine or concentrated saline and calculating the difference from total lipolytic activity [38], inhibition of the hepatic lipase by specific antiserum [31], or chromatographic separation of two enzymes [39]. In classic lipoprotein lipase deficiency, patients have marked triglyceridemia and virtually always have clinical symptoms before puberty, and they have defective enzyme activity in every tissue studied [40]. The enzyme may be measured in adipose tissue obtained by needle aspiration, which is of advantage because it does not contain hepatic lipase, but the assay must be done immediately thereafter. In patients with classic deficiency, the activity in adipose tissue is defective whether the patient is in the fed or fasted state [41].

Some individuals have been observed in whom there was a partial deficiency of the enzyme [40]. A form of familial dominantly inherited hyperchylomicronemia has been reported in which there was a circulating inhibitor of lipoprotein lipase activity [42]. Another patient has been described [43] in whom a transient deficiency of lipoprotein lipase led to an attack of acute pancreatitis.

The enzyme is a homodimeric glycoprotein with identical 60 kDa subunits [44]. In many patients with deficient enzyme activity, an immunochemically detectable protein is present, but a few patients have had no lipase protein [45]. The first of these was in a patient with no enzyme activity or cross-reactive material who had a 6 kb deletion involving exons 3–5 on one allele and a 2 kb deletion in exon 6 on the other [46, 47]. No mRNA could be found in adipose tissue.

The cDNA for the human enzyme codes for a mature protein of 448 amino acids. There is alternate splicing in adipose tissue, which produces two mRNAs [48, 49]. The mRNA is found in muscle, kidney, adrenal, intestine, and neonatal liver, as well as adipose tissues, but it is not found in adult liver.

Since the patient with the 6 kb deletion, a number of mutations has been identified, a majority of them missense [8, 50–52], but coding for very reduced or absent enzyme activity. A point mutation in exon 5 was found to account for the majority of alleles in the French-Canadian population, where prevalence is the highest in the world [22, 53, 54]. A C-to-T transition at nucleotide 875 led to a change from proline 207 to leucine. Dot blot analysis is available and a restriction site is available for analysis. G188E was common in Europe. Missense mutations have been found in 28 instances. Stop-codons have been produced by five single-base changes. A 3 kb deletion in exon 9 has been reported [55] and two splice site defects have occurred in intron 2 [56, 57]. The majority of missense mutations have been in exon 5, and in exons 4 and 6, areas of considerable homology among lipases. Some mutations have converted hydrophobic residues to less hydrophobic amino acids. Among mutations found in Japanese, a novel complex deletion insertion mediated by repetitive Alu elements led to the elimination of exon 2 [8]. Direct sequencing of the coding region of the LPL gene has detected some 97 percent

of those with LPL deficiency. Mutations are also detectable by deletion/duplication analysis.

Phenotypic expression in heterozygotes was reported [58] in a pedigree of a proband homozygous for a mutation, G-to-A at position 818 leading to a glycine 188 to glutamic acid substitution which resulted in an immunoreactive but nonfunctional enzyme. Heterozygotes had increased plasma triglyceride, VLDL cholesterol, and apolipoprotein B and decreased LDL and HDL cholesterol, clearly distinguished from noncarriers only after 40 years of age.

Differential diagnosis – apolipoprotein

C-II DEFICIENCY

A distinct molecular abnormality in the lipoprotein lipase enzyme complex has been defined as a deficiency in the apolipoprotein C-II (apoC-II) activator of the complex [59–65]. These patients tend to present clinically later than those with classic lipoprotein lipase deficiency, in post-adolescence or adult life. The nature of the defect was suggested in the first patient who had displayed hyperlipemia and no activity of lipoprotein lipase, when the concentration of triglycerides fell sharply following a transfusion of blood for anemia. It was demonstrated that his plasma completely lacked apoC-II.

Patients with this disorder have had abdominal pains and pancreatitis. None have been described with xanthomas or hepatomegaly [62]. A few have had splenomegaly, and about half have had anemia. The pattern of hyperchylomicronemia is the same as in classic lipoprotein lipase deficiency [61]. The deficiency has most often been documented by the assay of lipoprotein lipase in the presence and absence of apoC-II or normal plasma. It can be assayed directly by electrophoresis of the tetramethylurea-soluble apoproteins on polyacrylamide gel.

The two molecularly defined genetic disorders, apoC-II deficiency and lipoprotein lipase deficiency, are rare. The most common causes of milky plasma are acquired and they are secondary to such disorders as diabetes, nephrosis, or alcoholism. Some have been associated with systemic lupus erythematosus, malignant histiocytosis, or lymphoma. In addition, there are clearly familial examples of hypertriglyceridemia and patients with typical symptomatic type I hyperlipoproteinemia in whom no molecular defect can be defined. Of 123 patients studied for hypertriglyceridemia, 110 were acquired, and eight fell into this latter category [33]. Only five had an abnormality in lipoprotein lipase, and these studies were carried out in the laboratory most of us rely on for assays for lipoprotein lipase.

In apoC-II deficiency, biochemical data are consistent with pedigree information and an autosomal recessive mode of transmission [63]. The gene for apoC-II has been mapped to chromosome 19 [64] and contains four exons spanning 3.3 kb [66]. Patients to date have been homozygous for a single mutation and are often products of a consanguineous mating. The nature of mutation has been defined in many of the small number of kindreds so far described [51]. In four, single-base change has led to a stop-codon [66–70], and in one there was a single-base substitution in the methionine initiation codon [70]. A donor splice mutation intron 2 [71] rounds out this group, none of whom had demonstrable apoC-II protein on immunoassay. Four frameshift mutations would be expected to lead to truncated proteins; two of them had no detectable protein [72–77]. In a patient with the homozygous C1118A change in the APOC2 gene, which resulted in a Y63X, the patient had lipid encephalopathy, fatty deposits on cranial MRI, neurologic impairment, and impaired mental development [78].

TREATMENT

The dietary restriction of the intake of fat has a dramatic effect in clearing hyperchylomicronemia and the avoidance of all of the manifestations of the disease. The argument for treatment is the substantial morbidity and mortality from pancreatitis. This should be eliminated along with the abdominal pains if concentrations of triglycerides are kept below 2000 mg/dL [79]. Most recommendations [29] are to keep levels below 1000 or even 750 mg/dL. It is generally agreed that symptoms will not occur at these levels. Both saturated and unsaturated fats must be restricted. Overall restriction should be to less than 15 percent of the calories from fats. In adults, diets containing less than 50 g of fat a day are usually sufficient. A value of 0.5 g/kg is useful in initiating therapy in children. An exception to restriction of fats is medium-chain triglycerides, which do not contribute to chylomicrons [80]. Diets extremely low in fat are well tolerated and consistent with normal growth and development (Figure 86.7).

Triglyceride levels should be studied throughout the day, not simply after an overnight fast. The regimen should ensure compliance around the clock. No single meal should contain more than 20 g of fat. At the same time, deficiency of essential fatty acids must be avoided. The management of an infant or child with type I hyperlipoproteinemia can be very difficult. Triglyceride levels may rise suddenly from a few hundred to several thousand mg/dL following a single fat-filled meal [3]. Agents known to increase concentrations of triglycerides, include alcohol, estrogens, including oral contraceptives [22], diuretics, isotretinoin, Zoloft, and β-adrenergic blockers. Extreme dietary fat restriction during pregnancy has resulted in normal offspring. In the patients followed for over 30 years [19] the threshold level of triglycerides to trigger pancreatitis appeared to reduce with time. Despite progressive reduction in the insulin response to a glucose load, plasma glucose levels and that of hemoglobin Alc were not diabetic. Antioxidant therapy has been reported [81] to reduce the frequency of pancreatitis. Experience of treatment with diets highly restricted in fat beginning at less than one year of age indicated no adverse effects on growth [22].

Figure 86.7 LS: A 12-year-old girl with lipoprotein lipase deficiency. She had had episodes of pancreatitis, but she was well despite a lifelong severe restriction of the intake of fat.

Management of the acute abdominal pain requires vigilance about the diagnosis of pancreatitis and recognition that amylase values may be normal. The treatment of pancreatitis should follow the usual conservative regimen, with the additional precept that fat should be eliminated. In a neonate with chylomicronemia and congestive cardiac failure, triglyceride levels of 38,000 mg/dL were reduced to normal by plasmapheresis, and cardiac function became normal [82]. The treatment of apoC-II deficiency should generally be the same as that of lipoprotein lipase deficiency. An episode of pancreatitis may be successfully treated in apoC-II deficiency by the infusion of normal human plasma.

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PART 12

LIPID STORAGE DISORDERS

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87

Fabry disease

MAJOR PHENOTYPIC EXPRESSION

Angiokeratomas of the skin, episodic pain in the extremities, hypohidrosis, corneal and lenticular opacities, postprandial pain and diarrhea, neuropathy, renal disease, coronary and cerebral vascular disease, accumulation of glycosphingolipids with a terminal galactose, and deficiency of ceramide trihexosidase (α-galactosidase A).

INTRODUCTION

Fabry disease was first described in 1898 independently by Anderson [1] in England and Fabry [2] in Germany. The latter name has become its designation [3], possibly because Fabry continued to publish information about his patient over a 32-year period [4]. Anderson and Fabry each recognized the systemic nature of the disease but, as dermatologists, their focus was on the cutaneous angiokeratomas by which the patient is so readily recognized. The disease is also known as angiokeratoma corporis diffusum universale [5–7]. It was first noted to be X-linked by Opitz and colleagues in 1965 [7]. The disorder was appropriately classified as a glycosphingolipidosis following the isolation and characterization by Sweeley and Klionsky [8] of the Fabry lipid as galactosylgalactosylglucosylceramide (Figure 87.1). The molecular defect was demonstrated by Brady and colleagues [9] as an inability to cleave the terminal galactose from this ceramide trihexoside; thus, the defective activity is ceramide trihexosidase (Figure 87.1). The defective enzyme was shown by Kint [10] by means of an artificial substrate to be an α-galactosidase. It is referred to as α-galactosidase A to distinguish it from the α-N-acetylgalactosaminidase which is deficient in Schindler disease and is also an α-galactosidase (B). The gene is on the X chromosome at Xq22.1 [11] and the disease is expressed as an X-linked dominant. The gene has been cloned and its sequence determined [12–13]. A large number and variety of mutations have been defined. Among 34 different mutations 76 percent were missense, 16 percent nonsense and the rest splice site and frameshift mutations [14].

CLINICAL ABNORMALITIES

The initial symptom is usually pain occurring often within the first ten years of life (Table 87.1). It may be excruciating, has a burning quality, and tends to occur intermittently [15]. Pain is most often noted in the fingers and toes, or the hands and feet. There may be associated tingling acroparasthesias [16, 17]. An attack may be brief or may last for weeks. It may be induced by exposure to extreme heat or cold, fatigue or emotional stress. It may be associated with elevated body temperature and an elevated erythrocyte sedimentation

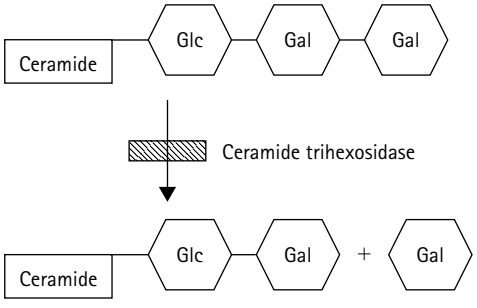

Figure 87.1 The enzyme ceramide trihexosidase is the site of the defect in Fabry disease. The reaction which cleaves a terminal galactose is an α-galactosidase.

Table 87.1 Clinical signs and symptoms of Fabry disease at different ages

Age	Signs
Childhood	Pain in extremities, fever, Fabry crisis
Adolescence	Angiokeratomas
Adulthood	Central nervous system symptoms
	Myocardial and pulmonary disease
Middle age	Renal failure, lymphedema

rate, and patients have been diagnosed as having rheumatoid arthritis or rheumatic fever [18, 19]. Degeneration of the interphalangeal joints may lead to deformities. Abdominal or back pain may suggest appendicitis or renal colic [20]. Narcotics may not provide relief. Crises of pain are self-limited, disappearing spontaneously, only to return later. Pains may decrease in frequency and severity with age, and some older patients have no history of pain. Patients may also have recurrent episodes of fever.

It is the appearance of the skin lesions in adolescence or later that usually leads to the diagnosis [5, 21]. These lesions are dark red punctate macules that do not blanch with pressure (Figures 87.2–87.4). They occur in clusters and may be mistaken for petechiae. With time, some become papular and may feel rough to touch. There is some tendency for bilaterally symmetric distribution. The areas of most common involvement are the scrotum and buttocks, but they are also seen on the hips, back, and thighs in a bathing-trunks distribution. The oral mucosa may also be involved. Microscopically, the skin lesions are angiectatic lesions in the dermis with keratotic build-up superficially. These angiokeratomas are not usually symptomatic, but occasionally large lesions on the scrotum may bleed. The hands or feet or just the tips of the toes or fingers may be bright red, and sensitive to touch. Lymphedema may also be seen in the legs. Hypohidrosis or even absence of sweating is another dermatologic manifestation of the disease. Sweat pores may appear

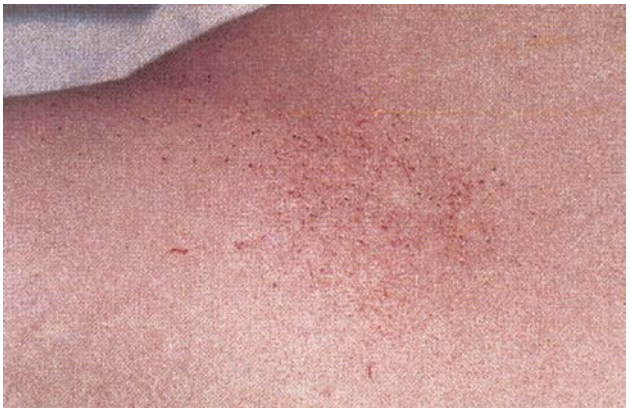

Figure 87.2 Angiokeratomas of the skin. These red-purple macules or maculopapules may feel hyperkeratotic. They are prominent on the hips, buttocks, and scrotum.

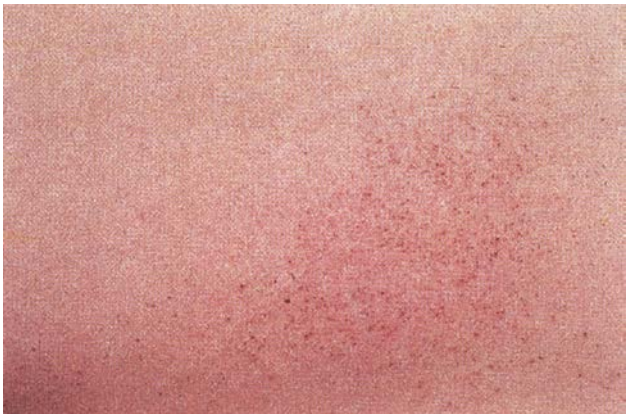

Figure 87.3 Angiokeratomas of the skin.

reduced. Patients are intolerant of heat and flush with exercise. One of our patients responded to hot weather by filling rubber boots with water and sloshing around in them, as well as soaking his head in cold water.

Ocular lesions [22] regularly include dilated tortuous venules of the conjunctivae (Figure 87.5). Similar dilatation may be seen in the vessel of the retinas. Corneal opacities develop in males and in some heterozygous females. The diagnosis can be made by slit lamp examination, in which the typical cream-colored interior, whorl-like opacities are visualized. Corneal opacities have been seen as early as six months of age [23]. Cataracts of the posterior capsule of the lens are pathognomonic [22]. The ocular lesions result from the deposition of glycosphingolipid and do not usually impair vision. As the disease progresses, the retinal changes of uremia may be found. Visual loss has been observed following central retinal artery occlusion [24]. Some

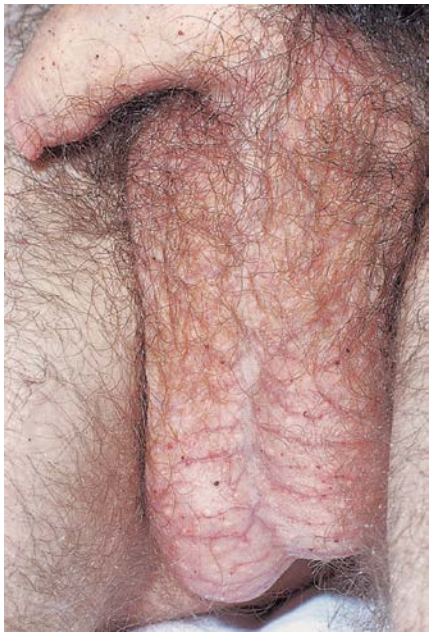

Figure 87.4 Angiokeratomas of the scrotum and penis of a 31-year-old patient.

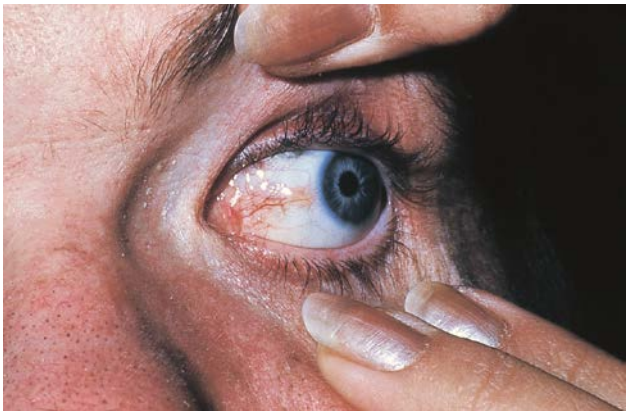

Figure 87.5 Tortuous, dilated, telangiectatic vessels of the conjunctiva. This man also had fine telangiectases of the facial skin.

patients display edema of the eyelids, in the absence of renal disease [22, 23]. The ocular manifestations of disease, especially cornea verticillata correlated with severe disease in pediatric patients [25]. Neurosensory hearing loss may develop [18].

Gastrointestinal manifestations may be prominent [26]. In some patients, they may be the only complaints for years. There may be postprandial pain or diarrhea. Infiltration of autonomic nerve cells and mucosal cells with lipid may interfere with peristalsis. Diverticula may develop, and rupture of a diverticulum is a surgical emergency.

The long-term complications of Fabry disease are consequences of the accumulation of glycosylsphingolipid in endothelial cells. The most regular concomitant of vascular dysfunction is chronic renal disease. The earliest manifestation is proteinuria, which usually occurs in the fourth decade. Hypertension is common. Examination of the urine may reveal red cells, casts, and birefringent lipid globules forming maltese crosses within and outside cells, best seen under polarizing microscopy. Renal function gradually deteriorates, leading to renal failure. This usually occurs by the fifth decade, but may occur as early as 21. The concentration of creatinine in the blood increases linearly with time [18, 27]. Polyuria is a manifestation of defective concentrating ability [28]. Prior to the development of programs of hemodialysis, many hemizygotes died before 40 years of age [29]. Renal disease as a cause of death in this disease has decreased while cardiac disease has increased [30]. Improvement in the management of renal disease appears to be the reason.

Cardiac manifestations of vascular disease include myocardial ischemia or infarction. Cardiac symptoms include shortness of breath, angina, or syncope resulting from arteriovenous (AV) block or ventricular outflow obstruction. Coronary occlusion or cerebral vascular disease often occurs before the age of 25 years [28, 31]. Cardiac enlargement and myocardial failure may result from infiltration of the myocardium or the valves with lipid [30, 31]. Arrhythmias are also common. The PR interval is shortened [32]. Echocardiography may show increased thickness of the interventricular septum and the posterior wall of the left ventricle [33].

Cerebrovascular manifestations may be transient ischemic attacks, strokes, seizures, hemiplegia, or aphasia. They result from infiltration and obstruction of cerebral vessels [34]. In a study of cryptogenic stroke almost 5 percent of 342 males had mutations in the Fabry gene [35]. Magnetic resonance imaging (MRI) or proton magnetic resonance spectroscopy (MRS) imaging may reveal cerebrovascular disease [34]. Some patients have had psychotic manifestations [36]. Abnormal cutaneous thermal sensation is common [34]. Elevated threshold for the detection of cold precedes that of warmth. Auditory and vestibular dysfunction increased with time [34].

Dyspnea on exertion is common, and airway obstruction may result from infiltration of bronchial epithelial cells [37]. Pulmonary function tests may show impairment. Other manifestations include lymphedema of the legs [38], priapism [39], and anemia [16]. Death usually results from uremia or from vascular disease of the heart or brain.

Heterozygous females have clinical manifestations with such frequency that the disease may be considered an X-linked dominant [40]. In a series of 20 heterozygotes none were asymptomatic [40]. All but two had pain or burning either chronically or in Fabry crises. Ten had lymphedema and 11 angiokeratomata. Typical corneal lesions were seen in 14. MRI revealed multifocal infarcts or white matter disease.

Multisystem disease and impaired quality of life was found to be common among 44 women [41] and acroparesthesia were the first reported findings is 74 percent.

In a series of 60 obligate carriers, studied largely by questionnaire, serious or debilitating consequences were found in 30 percent and hypohidrosis in 33 percent, while pains occurred in 70 percent [41]. Cardiac involvement, especially left ventricular hypertrophy and valvular abnormalities, were found to be common in a series of 35 female patients, and they progressed with age [42].

The pathology of Fabry disease consists of the widespread deposition of glycosphingolipid. Vacuoles are seen in a wide variety of cells, especially the endothelium of the blood vessels [43, 44]. Electronmicroscopy reveals a concentric or lamellar structure of the lysosomal inclusions.

In the Fabre outcome survey [45] of 1279 female and 1059 male patients diabetes increased with age. Patients under 50 years tended to have been diagnosed by nephrologists, while those diagnosed over 50 years were found by cardiologists.

GENETICS AND PATHOGENESIS

Fabry disease is an inborn error of glycosphingolipid metabolism transmitted in an X-linked character [7] which is far from fully recessive; the level of expression in the heterozygote ranges from asymptomatic to severity equal to that of the hemizygote.

The defective activity of α-galactosidase leads to the accumulation of glycosphingolipids that have a terminal α-galactosyl moiety [8, 46]. The most prominent is ceramide trihexoside (Figure 87.1), which is also known as globotriasylceramide [Gal(αl→4)Gal(β1→4)Glc(β1-1') ceramide]. Galabiosylceramide, a compound in which ceramide is linked to two galactose moieties, is also found in large quantities in tissues such as kidney [23]. The blood group B antigenic glycosphingolipid also contains a terminal galactose and, hence, it accumulates in Fabry patients with B or AB blood types [47]. Changes in concentration of globotriaosylceramide did not appear to be useful biomarkers for progression disease manifestations [48].

Globotriaosylceramide and globotriaosylsphingosine were dramatically increased in plasma of affected males [49]. Levels were reduced by therapy. Monitoring was recommended. Urinary globotriaosylceramide (Gb3), the substrate of the deficient enzyme was found to correlate with the types of mutations in 110 patients [50].

The basic defect in Fabry disease is an inability to degrade these glycosphingolipids because of defective activity of the enzyme that catalyzes the hydrolysis of the terminal galactose moieties [9]. The activity of α-galatosidase in most hemizygous males is less than 3 percent of normal, but as much as 20 percent of normal activity has been observed [51]. Studies with antibody against the enzyme have usually shown no cross-reactive material [52, 50]. α-Galactosidase is synthesized as a 50 kDa precursor protein, transported to the lysosome in a mannosphosphate receptor-dependent processing and cleaved to a mature 46 kDa enzyme [53]. Study of this sequence in cells of classic Fabry patients has indicated considerable variation: no enzyme precursor synthesized; precursor, but no mature proteins as a consequence of protein instability; and normal-appearing mature protein with no catalytic activity [53].

The gene for α-galactosidase has been localized to Xq22.1 [11]. X-linked inheritance was first established by pedigree analysis (Figure 87.6). Two populations of cells – one with normal galactosidase activity and the other defective – were shown by cloning of cultured fibroblasts [54]. The cDNA for the gene for α-galactosidase has been cloned and sequenced [12], and this has permitted delineation of the nature of a number of mutations. The gene has seven exons. Major gene rearrangement detected by Southern hybridization included five deletions and duplication [55]. A number of smaller deletions and insertions have been identified, many of which led to frameshifts and premature termination. Most mutations in Fabry hemizygotes are not detected in this way. Mutations altering the processing of the mRNA transcript have been observed. Single nucleotide missense mutations have been identified in a majority of families [55–57], and most have been found only in one single family. However, a high frequency of mutation was observed at 14 CpG dinucleotides in the coding sequence. More than 300 mutations have been found in patients with Fabry disease [58]. Among them two novel mutations, 1277delAA (del2) and 1284delACTT(del4) in the 3′ terminus, obliterated the termination codon and generated multiple transcripts, most of them inactive [58]. Among 110 patients [50] the mutations were 76 percent missense 16 nonsenses and 3 percent each frameshift and splice site.

Fabry disease is the most common lysosomal storage disease after Gaucher disease. The prevalence of heterozygous carriers in the United Kingdom was estimated at 1 in 339,000 females [41]. Heterozygote detection has been carried out by enzyme assay of cultured fibroblasts after cloning [52] and cell sorting, but these methods are not practical for clinical use. The assay of the enzyme in individual hair roots [59] is more convenient but still labor intensive. In a family in which the mutation has been identified, targeted analysis can be employed for precise identification of heterozygosity.

Prenatal diagnosis has been accomplished by the demonstration of deficient α-galactosidase activity in cultured amniotic fluid cells [60]. A microtechnique for α-galactosidase has been developed for prenatal diagnosis, which requires small numbers of cultured amniocytes [61]. Diagnosis has also been made prenatally by chorionic villus sampling [62]. The identification of the molecular nature of mutation in a family permits this molecular technique to be used for prenatal diagnosis.

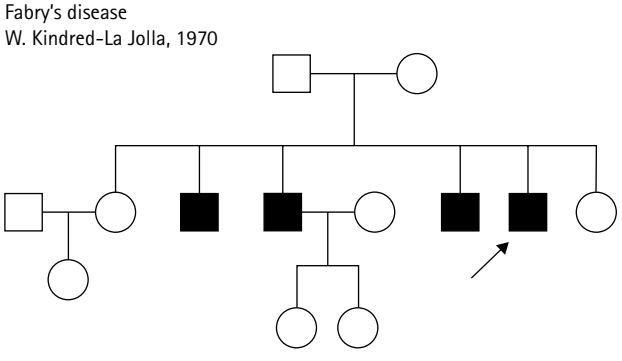

Figure 87.6 Pedigree of a family with four affected males. The pattern is of X-linked recessive inheritance.

TREATMENT

Enzyme replacement therapy has become the standard of care for patients with Fabry disease (63) It is ideally begun prior to the onset of symptoms, at least after 18 years of age in males. Products include agalsidase alfa (Replagal, Shire) and agalsidase (Sanofi Genzyme). The former is given (0.2 mg/kg) every other week, by intravenous injection. The latter is given (1 mg/kg) every other week intravenous.

Renal biopsy has documented [64] the disappearance of GL31 lipid over five years of treatment.

Prevention and amelioration of the painful crises of the disease have been difficult, but diphenylhydantoin has

been shown to be helpful; chronic low dosage has been employed (200–300 mg qd) [17]. Carbamazepine may also be helpful, and the combination of the two drugs may be particularly useful [63]. Gabapentin (Neurontin) has been recommended for this purpose [64]. Doses employed in adults have ranged from 100 mg bid to 300 mg bid, or 15–60 mg/kg in children. Neurotropin, an extract of inflamed skin of vaccinia inoculated rabbits was reported to be as effective as carbamazepine in the usual leg pains; neither were effective in episodic colicky pain, but treatment with both eliminated it [65].

Chronic hemodialysis has been the mainstay of management of renal failure. Many patients have received kidney transplants following renal failure [65–68]. This solves the problem of renal failure, but does not alter the accumulation of lipid in other tissues. Some transplanted patients have survived long enough to die of cardiac disease. Enzyme replacement therapy with purified α-galactosidase [69] has been extended to trials with recombinant human enzyme, which have demonstrated safety and efficacy [70–75]. It is clear that treatment reverses the storage in lysosomes, the causes of clinical disease. A ten-year study of 52 patients in the Fabry registry treated with agalsidase yielded 81 percent who did not experience a severe event during treatment and 94 percent who were alive [65]. The younger the age of initiation of treatment, the better was the result. Early treatment was also emphasized [66] in a report of long-term therapy, which also warranted that premature death still occur in treated patients.

Activation of mutant enzymes by compounds such as 1-deoxygalactonojirimycin are being explored in novel approaches to the treatment of glycosphingolipidoses [76, 77]. This compound is an inhibitor of lysosomal α-galactosidase but, in low doses, it serves as an activator increasing activity in mutant enzymes up to 14 times. These compounds have been referred to as chemical chaperones, because they accelerate transport and maturation of the enzyme molecule. Chaperone therapy with migalastat was found to confer results equal to enzyme replacement therapy [78, 79]. Renal, cardiac, or cerebrovascular events continued to occur in both groups but results were encouraging for mono therapy. A pharmacogenetic test in which mutations were expressed in HEK cells identified mutations amenable to treatment with migalastat [80].

Another approach is substrate deprivation. Compounds such as D-*erythro*-1-ethylenedeoxyphenyl-2-palmitylamino-3-pyrrolidino-propanol (d-t-EtDO-P4) reduce accumulation of globotriosylceramide in tissues of murine Fabry models [81] by inhibiting the sphingolipid glucosyltransferase involved in their synthesis. A prevalent c.639+919G>A mutation which leads to pathogenic insertion of a 57 bp pseudoexon, could be blocked by a splice switching oligonucleotide (SSO), effectively restoring normal splicing, and providing the genetic alternatives [82].

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88

Tay-Sachs disease/hexosaminidase A deficiency

MAJOR PHENOTYPIC EXPRESSION

Infantile cerebral and retinal degeneration with cherry red macular spots, hyperacusis, macrocephaly, storage of GM_2 ganglioside in the brain, and deficiency of hexosaminidase A.

INTRODUCTION

Tay-Sachs disease has been described as the prototype of the lysosomal storage disorders [1]. It represents a paradigm for the success of research in biochemical genetics not only in providing precise molecular understanding of the nature of disease but also in the practical community-based control of a genetic disease.

The disease was first described by Tay [2] in 1881 in an infant in whom a cherry macular spot was associated with delayed development. Sachs [3] defined the clinical entity, which he called a familial amaurotic idiocy [4]. The enzymatic defect was discovered in 1969 by Okada and O'Brien [5]. The deficiency in hexosaminidase A results in a failure to cleave the terminal N-acetylgalactosamine (GalNAc) from the GM_2 ganglioside (Figure 88.1). The development of methodology for the rapid, relatively easy quantification of the A isozyme has permitted accurate identification of heterozygous carriers of the gene and prenatal diagnosis, permitting a public health approach to human genetics and the virtual prevention of the birth of affected children in the population at highest risk [6, 7].

The various disorders of ganglioside GM_2 storage are summarized in Table 88.1. All are progressive cerebral degenerative diseases. The cherry red macular spot is a prominent feature in all of the early infantile presentations, all of which are fatal in infancy. All of these diseases are autosomal recessive. Neuronal lipidosis is a common histologic feature and results from the storage of ganglioside. Deficiency of lysosomal hydrolase activity provides in each a molecular explanation for the disease in which storage of GM_2 ganglioside results from failure to cleave its terminal GalNAc. There are three types of GM_2 gangliosidosis. Sandhoff disease (Chapter 89) has been referred to as the O variant to indicate that neither hexosaminidase A nor hexosaminidase B is active. In this classification, Tay-Sachs disease was termed the B variant, since only the B isozyme is active; in what was called the AB variant both enzymes are active and the defect is in the GM_2 activator (Chapter 93).

Hexosaminidase A is a heterodimer containing the α and β subunits. Hexosaminidase B is composed of two β subunits. Cultured cells of patients with Tay-Sachs disease lack the α chain. The genes for the α and β chains have been cloned and the locus for the α chain and for Tay-Sachs disease is on chromosome 15q23 [8]. The gene is common in Ashkenazi Jews. A considerable number and variety of mutations have been described, most in patients with the classic infantile phenotype [9, 10]. The most frequent mutation in the Ashkenazi Jewish population is a four-nucleotide insertion in exon 11, which introduces a frameshift and a downstream premature termination signal that results in a deficiency of mRNA.

CLINICAL ABNORMALITIES

Patients with Tay-Sachs disease appear normal at birth, although storage of GM_2 ganglioside has been demonstrated even in the fetus [11]. Infants continue to appear alert and healthy until about six months of age. The onset of clinical disease may be between birth and ten months of age [12]. The earliest clinical manifestation may be an exaggerated

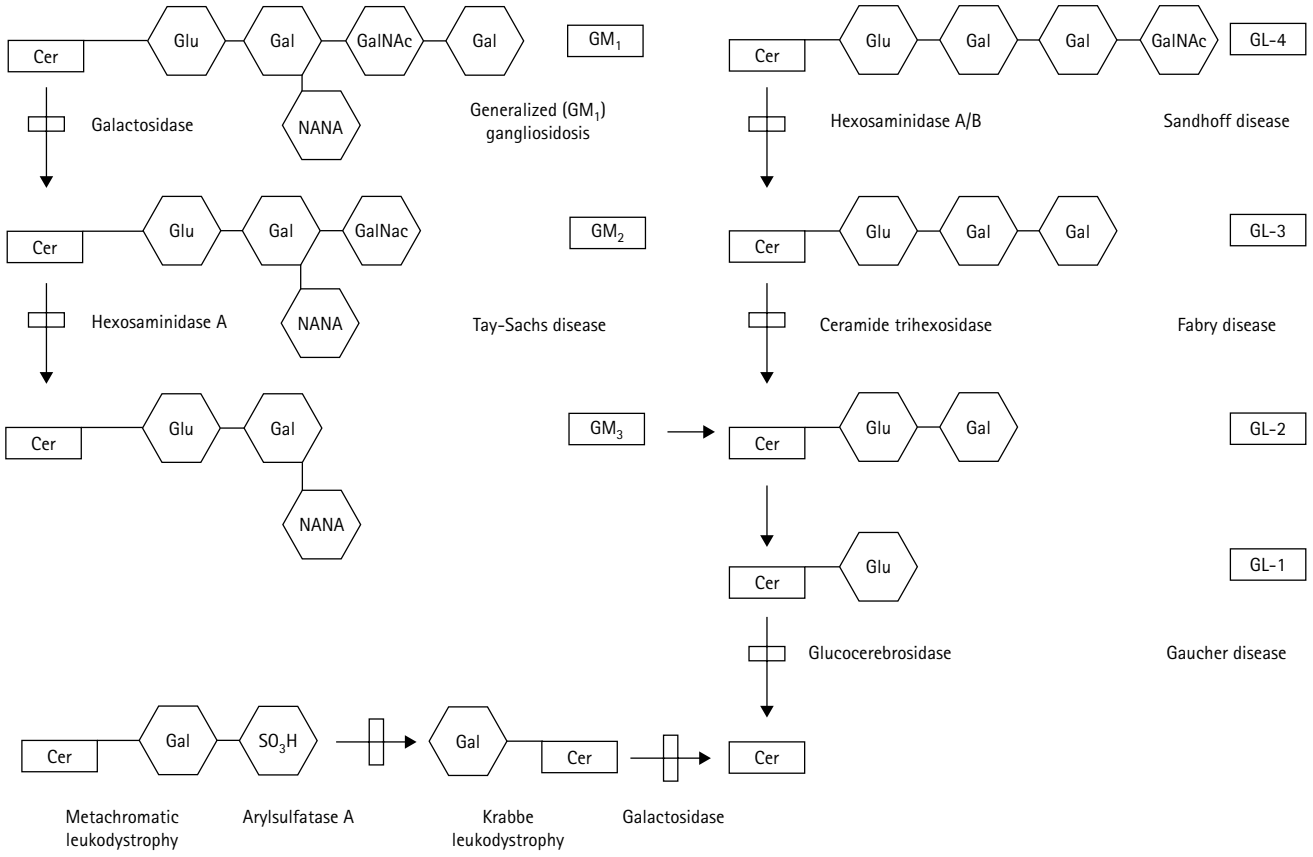


Figure 88.1 Metabolic pathways of glycosphingolipid metabolism. The site of the defect in a number of conditions is illustrated. That of Tay-Sachs disease is in hexosaminidase A, which catalyzes the removal of N-acetyl-galactosamine from the GM_2 lipid to produce GM_3. Cer, ceramide; Glu, glucose; Gal, galactose; NANA, N-acetyl-neuraminic acid; and GalNac, N-acetylgalactosamine.

Table 88.1 GM_2 ganglioside storage diseases

Disorder	Age at onset	Age at death (years)	Enzyme defect	Carrier detection	Prenatal diagnosis
Tay-Sachs disease	3–6 months	2–4	Hexosaminidase A	+	Established
Sandhoff disease	3–6 months	2–4	Hexosaminidase A and B	+	Established
AB variant	3–6 months		Activator	+	Possible
Juvenile GM_2 gangliosidosis	2–6 years	5–15	Hexosaminidase A	+	Possible

startle response to sound, in which the arms and legs extend. This is usually present by one month of age, but it may not be appreciated early, since it can be seen in some normal babies, usually disappearing in about four months. In contrast, in the baby with Tay-Sachs disease this hyperacusis becomes more prominent. It is brought on even by very gentle sound stimuli. It may be accompanied by clonus.

Parents may notice motor weakness as the first clinical sign. By eight months of age, the baby may look sleepy or less alert. The infant may begin to sit less well or to begin to lose head control. Physical examination at this stage reveals hypotonia. This is progressive. By one year of age, few of these patients can sit without support. The usual developmental milestones are lost or never attained. There may be nystagmus and a fixed, staring or roving gaze. Examination of the fundus reveals the typical cherry red spot in the macula (Figure 88.2). This is usually present as early as two months of age and has been demonstrated by retinal photography as early as the first days of life. In looking for this, it is important to remember that the white degeneration of the macula is larger and more impressive than the red foveal spot in the middle. Together, they look very much like a fried egg. Lipid storage in the ganglion cells obscures the choroidal vessels behind. In the fovea, where ganglion cells are few in number, vascularity of the choroid is seen as the red spot. With time, the spot may become darker or brownish in color. Opaque white streaks may develop along the vessels.

Cerebral and macular degeneration is rapidly progressive. The infant becomes blind, rigid, and

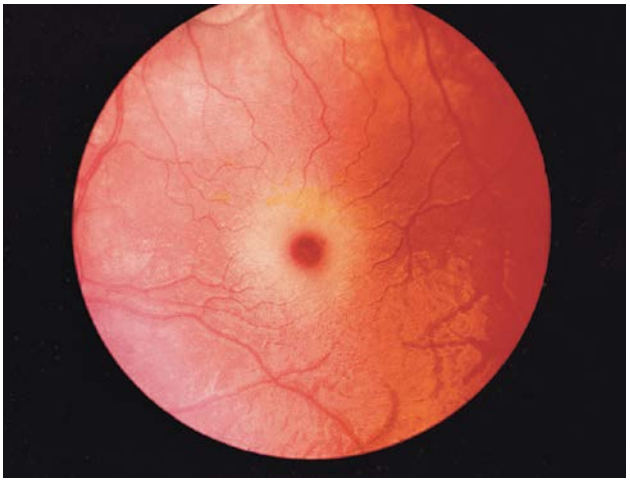


Figure 88.2 The cherry red spot. Photograph of the fundus of an infant with Tay-Sachs disease.

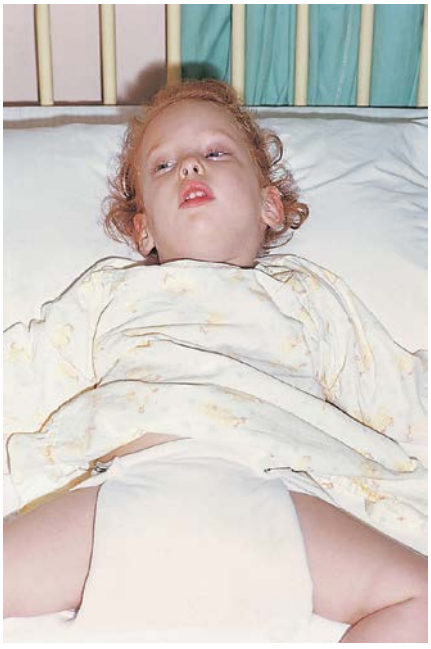


Figure 88.3 AS: A two-year-old patient with Tay-Sachs disease. He was blind and decerebrate at this time. Hexosaminidase A activity was absent.

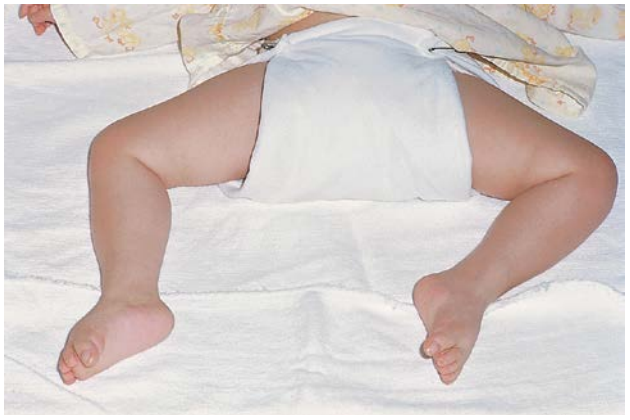


Figure 88.4 The lower extremities of the same patient were flaccid.

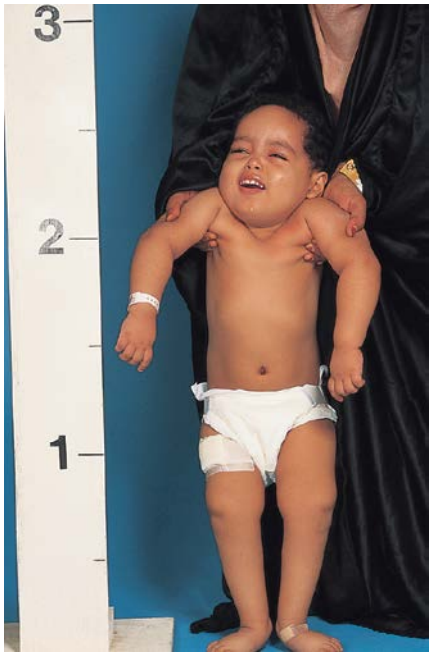


Figure 88.5 MAS: A two-year-old boy with Tay-Sachs disease. He was hypotonic but had increased deep tendon reflexes and positive Babinski responses. He had hyperacusis and was blind.

decerebrate by 12–18 months (Figure 88.3), and usually must be fed by tube because swallowing is ineffective. The extremities may be flaccid (Figure 88.4), but muscle tone is usually increased, and there is hyperreflexia (Figures 88.5 and 88.6) and opisthotonos. Convulsions and myoclonic jerks are common. Seizures almost invariably occur after one year of age, but they are not difficult to control with anticonvulsant medication. Electroencephalograph (EEG) abnormalities are relatively mild but become progressive after the first year [13]. The electroretinogram is normal, but visual evoked potentials disappear. Thalamic hypointensity has been reported [14] as a clue to the diagnosis.

Patients often have a doll-like facial appearance (Figure 88.3) with clear, translucent skin, long eyelashes, fine hair and delicate pink coloring. After about 15 months, the head size usually enlarges. By this time there is decerebrate posturing, difficulty with swallowing and secretions, and vegetative unresponsiveness. The brain weight at the time of death may be 50 percent heavier than normal. This is a consequence of glial proliferation and of lipid storage. There is no hepatosplenomegaly or other peripheral evidence of storage disease.

Death usually results from aspiration and pneumonia; usually by two to four years of age. Pathologic changes are restricted to the nervous system, where the neurons are

Figure 88.6 FHS: A 16-month-old girl with Tay-Sachs disease. She was hypotonic but hyperreflexic and had bilateral ankle clonus and positive Babinski responses. Pupils reacted poorly to light, and she had bilateral cherry red spots.

swollen, or "ballooned", displacing the nucleus toward the periphery [15]. This picture may be seen in neurons of the autonomic system and rectal mucosa as well as in the cerebral cortex. "Meganeurites" have been described among cortical neurons [16]. Electron microscopy of the neuron reveals lamellar membranous cytoplasmic bodies [17]. These inclusions are round concentric layers of accumulated ganglioside cholesterol and phospholipid in lysosomes. Pathologic changes can be demonstrated by electron microscopic study of biopsied skin, [18]. As axonal degeneration proceeds in the brain, there is secondary demyelination and cortical gliosis.

A certain amount of genetic heterogeneity has been established among GM_2 gangliosidoses [19–21]. The first to be appreciated was Sandhoff disease (Chapter 89), in which there is deficiency of both hexosaminidases A and B and a phenotype indistinguishable from that of Tay-Sachs disease. This is also true of GM_2 activator deficiency [22].

A more indolent phenotype referred to as juvenile GM_2 gangliosidosis has an onset at about two years of age with ataxia and incoordination. Speech is lost and deterioration is progressive to spasticity and decerebrate rigidity. Activity of hexosaminidase A is deficient but not to the degree seen in Tay-Sachs disease [20, 23]. A less severe form of GM_2 gangliosidosis has been referred to as adult or chronic GM_2 gangliosidosis [24]. This and the juvenile phenotype represent parts of a spectrum of genetically determined variants, ranging from the classic infantile Tay-Sachs phenotype to adult disease that progresses so slowly intellect is hardly affected. The advent of molecular biology and the very extensive documentation of mutation make most of these clinical classifications obsolete.

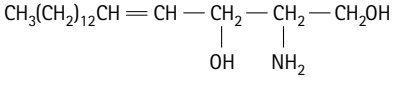
Prominent features in variant patients are distal or proximal muscle atrophy, pes cavus or foot drop, as well as spasticity, dystonic movements, dysarthria, and ataxia. The overall picture may be reminiscent of spinocerebellar degeneration [25, 26]. Some patients [27] have been clinically normal when first diagnosed on the basis of low enzyme activity, but later have developed difficulties of speech and gait. Others have been studied [28, 29] in whom clinical manifestations have not been observed. Psychotic disease has been common in adult onset GM_2 gangliosidosis [30, 31]. Response to antipsychotic medication has been poor. Some patients have been observed with problems of supranuclear gaze [32], raising the differential diagnosis of Niemann-Pick type C disease (Chapter 92). Eye movements have been studied in late onset Tay-Sachs disease [33]. Decelerations were earlier and faster and smooth pursuit was impaired.

An assay has been developed [23] in which fibroblasts of the various phenotypes have had activities of hexosaminidase A that correlated well with the clinical picture. In this assay, patients with Tay-Sachs disease displayed 0.1 percent of control activity, the late-infantile or so-called juvenile 0.5 percent, and the adult GM_2 gangliosidosis 2–4 percent. The clinically asymptomatic individuals with low hexosaminidase activity had 11–20 percent of control activity.

GENETICS AND PATHOGENESIS

Tay-Sachs disease is transmitted as an autosomal recessive disease. The gene frequency in Ashkenazi Jews has been calculated to be approximately one in 30 [6]. This would predict an annual incidence of one in 4000 births with Tay-Sachs disease among parents from this population. These frequencies were so high that it became practical to undertake programs of prevention through heterozygote detection. Gene frequency in non-Jews has been calculated to be one in 300 [6]. The disease is also common in some isolates in Switzerland [34] and in French descendants in Eastern Quebec and Southern Louisiana [35].

The GM_2 ganglioside stored in Tay-Sachs disease is an acidic glycosphingolipid with a terminal hexosamine, GalNAc (Figure 88.1) [36–39]. The ganglioside, which is normally present in very small amounts, is increased 100 to 1000 times in Tay-Sachs disease. The sphingolipids all contain the long-chain base sphingosine, which has the following structure:



This compound is acylated with long-chain fatty acids on the amino group on carbon 2 to form ceramide, which makes up the base unit of all of the sphingolipids. In the

gangliosides, as well as in the cerebrosides and glycolipids, a sugar is linked glucosidically to carbon 1. In the parent ganglioside, GM_1, the glycolipid that accumulates in generalized gangliosidosis, ceramide is linked successively to glucose; to galactose, to which N-acetylneuraminic acid is attached; to N-acetylgalactosamine; and to galactose. This terminal galactose is cleaved by a β-galactosidase, which is defective in generalized GM_1 gangliosidosis, to yield the Tay-Sachs lipid, GM_2 [37]. This is normally converted to GM_3 by cleavage of the terminal GalNAc. The amounts of GM_2 storage in the brain in variant patients tend to be less than in Tay-Sachs disease [40, 41].

The defect in hexosaminidase A represents a failure to hydrolyze this terminal aminosugar from the GM_2 ganglioside. It was first demonstrated by Okada and O'Brien [5] by starch gel electrophoresis which separated hexosaminidase activity into two components, designated A and B, the former (A) of which was absent in patients with Tay-Sachs disease. The A enzyme is more heat-labile and negatively charged than the B isozyme [39]. The diagnosis is made by measuring total and heat-stable hexosaminidase activity in serum, using artificial methylumbelliferyl N-acetylgalactosamine or N-acetylglucosamine substrate whose product of cleavage is the fluorigenic 4-methylumbelliferone [42]. The heat-labile enzyme is hexosaminidase A, and its activity is represented by the difference in activity before and after denaturation. The enzyme can also be measured in freshly isolated leukocytes, tears and cultured fibroblasts or amniotic fluid cells [43]. In Tay-Sachs disease, the activity of hexosaminidase A is virtually zero [5, 21, 44]. Assays have also employed [20, 21] the natural substrate GM_2 ganglioside.

Cross-reacting material (CRM) was demonstrated using antibodies prepared against human placental hexosaminidase A and the α-subunit of kidney and liver extracts of some patients with Tay-Sachs disease [45], but most are CRM-negative. The gene for the α subunit of hexosaminidase A contains 14 exons over 35 kb and 5′ regulatory elements (TATA) and 3′ untranslated areas [46]. Very many mutations have been documented [6, 10, 47–51] spanning all 14 exons. Twenty-one of these occur at CpG dinucleotide sites, which are known to be mutagenic hot spots and which account for more than one-third of human polymorphisms and disease mutations [48, 49]. Even within the classic Tay-Sachs infantile phenotype, almost 100 different mutations have been reported [6, 9, 10]. Deletions in sections, frameshifts, and stop codons are found in this phenotype.

Two mutations account for 93 percent of the mutant alleles in the Ashkenazi Jewish population of North America (Table 88.2) [9, 47]. In addition to the 4 bp insertion, 1278ins4, the other common deletion in this population is a splice site inversion in intron 12. A G-to-C change in the first nucleotide of the intron leads to several abnormally spliced mRNAs. The other common mutation is the French-Canadian mutation, a 7.6 kb deletion in exon 1 and flanking sequences in a population in which the frequency of the disease has been similar to that of the Ashkenazi Jewish population [52].

Table 88.2 Mutations in the hexosaminidase A gene in Tay-Sachs disease

Mutation	Population	Frequency
+TATC 1278ius4	Ashkenazi Jewish	80%
+IVS 12 (G→C)	Ashkenazi Jewish	15%
G269S	Ashkenazi Jewish	Most late onset
910 del TTC	Moroccan Jewish	Most of that population
−IVS 5-1G→T	Japanese	Most of that population
+IVS 7 (G→A)	French Canadian	Rare
+IVS 9+1(G→A)	Celtic, French, Pennsylvania Dutch	Rare
DPhe304 or 305	Moroccan, Jewish, Irish, French	Rare
−IVS4 (G→T)	Armenian, Black	Rare
C deletion 1510	Italian	Rare
A→G, exon 1	American Black	Rare
G436 deletion (exon 4)	American Black	Rare
C→T 409 (exon 3)	American non-Jewish, Caucasian	Rare

Assembled from data reported by Kaback et al. [6].

All other mutations are rare and have generally been found in compounds except in consanguineous families. The D Phe 304 or 305 represents deletion of one of two adjacent phenylalanine moieties [49]. The mutation found in two unrelated black American families [50] was an interesting one in which a G-to-T transversion in the invariant AG of the acceptor splice site of intron 4 interfered with splicing. An A-to-G transition in exon 1 found in a black American family changed the initiating methionine to a valine. Of the last two mutations in Table 88.2, C to T409 creates a termination codon, and deletion of G436 frameshift leads to a termination codon. Some interesting mutations interfere with the assembly or processing of a synthesized α subunit. R504C and R504H are secreted, and not retained in early compartments but fail to associate with β subunits to form the enzyme [49, 53].

The mutations associated with the later-onset phenotypes of GM_2 gangliosidosis have generally been single-base substitutions, leading to a single amino acid change [6]. Many have been found in compounds. In one [54], a mutation, G570A, led to alternate splicing in which a certain amount of normal mRNA was made, while in most of the mRNA exon 5 was missing. The adult-onset phenotype in Jewish populations results in a glycine 269 to serine change [55].

Heterozygous carriers of the gene have intermediate activities of hexosaminidase A in their serum or plasma [42]. These values average 65 percent of those of normal. Screening for heterozygosity should be done prior to the

Table 88.3 Prevention of Tay-Sachs disease (1971–92), showing >90 percent reduction in the disease in Jewish population (1970–93)

Group	Number	
Total screened	9.53×10^6 (seven countries)	
Carriers identified	36,418	
Couples at risk	1056	
Pregnancies monitored	2415[a]	
Affected fetuses	469	
Aborted	451	
Normal offspring born	1881	
Births per year with Tay-Sachs:		
Prior to 1969	100 (US and Canada)	(80% Jewish)
1980	13	(80% non-Jewish)
1985–92	3–10	(85% non-Jewish)

[a] Prior offspring as well as heterozygote screening (1969–1992).

development of pregnancy, since this may cause a false positive result in the serum assay. The issue can be resolved by assay of leukocytes.

The heat denaturation assay has been automated and employed in mass screening for heterozygotes throughout the world [56–58]. Such programs have shown that the disease can be virtually eliminated in those at highest risk [6]. Between 1971 and 1992, almost a million people were screened for heterozygosity in 17 countries (Table 88.3). Of these, over 36,000 carriers were detected and 1000 couples at risk were identified because both were carriers. Considering couples identified by screening and also those identified because of prior offspring, 2416 pregnancies were monitored and 469 affected fetuses were found, of which all but 18 were aborted. More important than the prevention of all these patients with Tay-Sachs disease, almost 2000 normal offspring were born to these couples at risk. When this program began, 50–100 infants with Tay-Sachs disease were born annually in the United States and Canada, 80 percent of whom were Jewish. In the past 20 years, the incidence has varied between three and ten annually, of whom 85 percent were non-Jewish. This represents a 90 percent reduction in the incidence of Tay-Sachs disease in the Jewish population of the two countries. A program of screening in Australia has emphasized the testing of high school students [59]. Carrier frequency was one in 25 Ashkenazi Australians.

Tay-Sachs disease can be detected prenatally [11]. The enzyme is reliably assayed in cultured amniotic fluid cells or chorionic villus material [6, 60]. Tay-Sachs is the metabolic disease most frequently diagnosed prenatally [6]. Early pitfalls or misdiagnoses have been eliminated by regular use of cultured amniotic fluid cells for assay, prior establishment that both parents are heterozygotes [61], and the use of ultrasonography to rule out the presence of twinning. Even so, there have been a few misdiagnoses, all attributable to such laboratory errors as mix-ups of samples. Risk of miscarriage is 1 percent or less [62]. Preimplantation diagnosis has been carried out [63].

Testing of potential heterozygotes by enzyme assay with synthetic substrates has uncovered the existence of pseudodeficiency genes that can result in a healthy person who has no hexosaminidase A activity in this assay. Approximately 35 percent of non-Jewish persons identified as heterozygotes by enzyme assay are pseudodeficiency carriers [64]. Many of the individuals have been identified to have a mutation substituting tryptophan 247 for arginine [65]. The existence of the pseudodeficiency allele makes it essential to do mutational analysis in couples at risk because both have been identified as carriers by enzyme analysis. There is no risk if one or both carry the pseudodeficiency allele. In families in which mutation has been identified, analysis for mutation can be employed for heterozygote detection and prenatal diagnosis [66].

TREATMENT

Specific treatment has not been developed. Skilled supportive care should be provided for the family, and the patient should be made as comfortable as possible.

Pyrimethamine has been found to increase hexosaminidase activity, slowing the progression of late onset Tay-Sachs disease [67]. Substrate reduction therapy has been used in mice with N-butyldeoxynojirimycin [68]. Sialidase has been used *in vivo* to deplete cultured Tay-Sachs cells of GM_2 ganglioside [69]. Miglustat, an inhibitor of glucosylceramide synthase, which catalyzes the first step in the syntheses of GM_2 ganglioside, is also under investigation [70]. Creation of a novel variant of HexA incorporating β subunit sequences and an adenovirus vector has been shown to degrade GM_2 storage in mice. Manipulation of cellular folding in certain variant HexA cells was found to alter mutant degradation [71].

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89

Sandhoff disease/GM_2 gangliosidosis/deficiency of Hex A and Hex B subunit deficiency

MAJOR PHENOTYPIC EXPRESSION

Progressive cerebral degeneration starting at six months of age, blindness, cherry red macular spots, hyperacusis, accumulation of GM_2 ganglioside, and deficiency of hexosaminidase A and B (Hex-A and Hex-B), resulting from mutation in the gene for Hex-B.

INTRODUCTION

The clinical phenotype of Sandhoff disease may be indistinguishable from that of Tay-Sachs disease (Chapter 88), but there may be hepatosplenomegaly in Sandhoff disease. The distinction between the two conditions was delineated by Sandhoff *et al.* [1] in 1968, in a patient who was unusual in that he stored ganglioside not only in the brain but also in other viscera, in contrast to patients with Tay-Sachs disease. The activity of total hexosaminidase was found to be deficient [1]. Hexosaminidase B is a glycoprotein homopolymer with four identical subunits; its structure is designated β2β2 [2, 3]. Hexosaminidase A is a heteropolymer of α and β subunits. Activity of the Hex-A and Hex-B isozymes are defective because of a defective β subunit. The disease has also been referred to as GM_2 gangliosidosis (variant O). The Hex-B gene is located on chromosome 5q13 [4]. Heterogeneity has been observed in the mutations in the gene for Hex-B [5]. Most mutations lead to the most severe infantile onset phenotype. The causative mutations in these patients tend to be deletions, nonsense mutations, or splice site mutations. The most common is a 16 kb deletion that includes the promoter, exons 1 to 5, and part of the intron [6].

CLINICAL ABNORMALITIES

It has often not been possible to distinguish Sandhoff disease from Tay-Sachs disease clinically [2, 3, 7–9]. The disorder tends to be suspected in non-Jewish patients with the Tay-Sachs phenotype. Individual patients appear normal at birth and appear to develop normally (Figures 89.1–89.9) until four to nine months of age, when signs of motor weakness and hypotonia begin to become evident.

Abilities that have been learned are progressively lost. These might include the ability to grasp objects or to sit, crawl, or hold up the head. Patients never learn to walk. Many of these infants have doll-like faces with long eyelashes and fine hair, pale translucent skin and pink coloring.

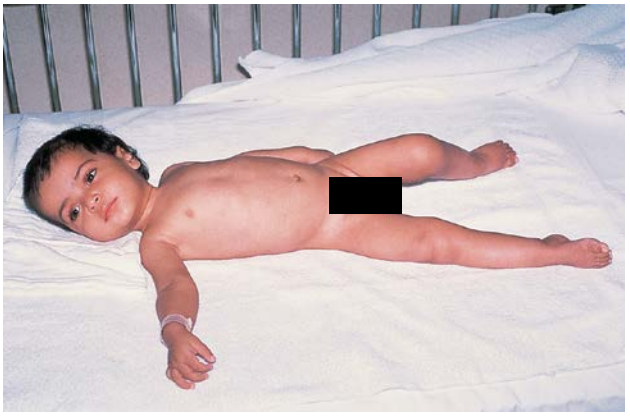

Figure 89.1 SO: A 13-month-old child with Sandhoff disease in the tonic neck reflex position. He had spasticity and was hyperreflexic. Babinski responses were positive bilaterally and he had ankle clonus. The neck was hypotonic.

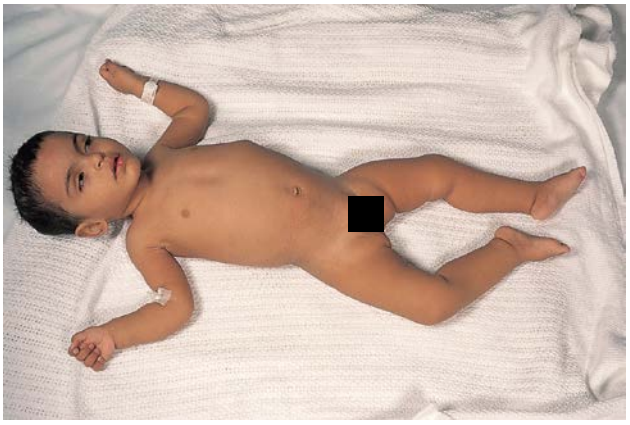

Figure 89.2 TO: There was no evidence of coarse features. Activities of Hex-A and Hex-B were virtually completely deficient.

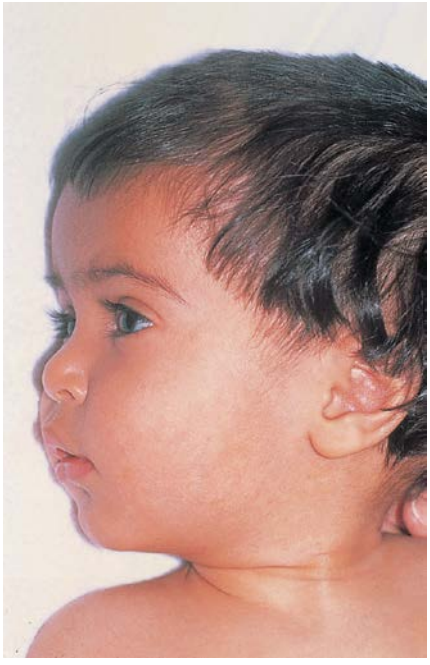

Figure 89.3 TO: At 18 months. Regression had begun after six months and all milestones were lost. She had an exaggerated startle to noise.

Cherry red macular spots (Figures 89.10 and 89.11) are seen bilaterally. Blindness is progressive and optic atrophy develops. Patients develop hyperacusis, or an exaggerated startle response to noise, which may be seen even quite early. The size of the head increases abnormally. Seizures are common; they develop some months after the onset of clear neurologic abnormality. They may be generalized or myoclonic. The electroencephalogram (EEG) also becomes progressively more abnormal. Visual loss is progressive and visual evoked potentials abnormal. Nystagmus was the initial sign in one patient [8]. Spasticity develops, and mental deterioration continues until the patient is rigid, decerebrate, and completely blind. Cardiac involvement and

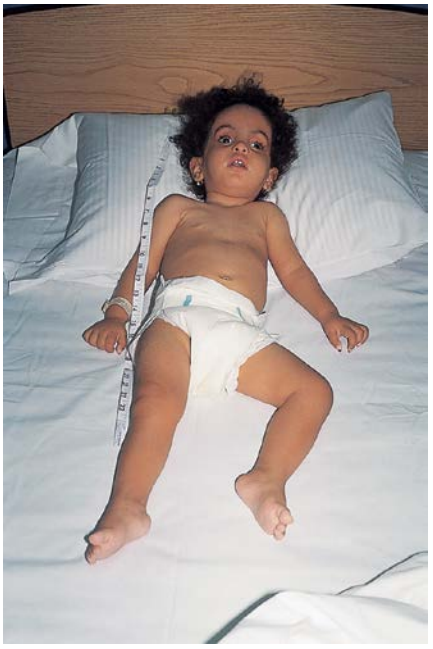

Figure 89.4 MO: A 17-month-old child with Sandhoff disease. She was flaccid and apathetic. Head circumference was increased and she had acoustic myoclonus to slight sound, as well as myoclonic and tonic seizures. Leukocyte hexosaminidase activity was zero.

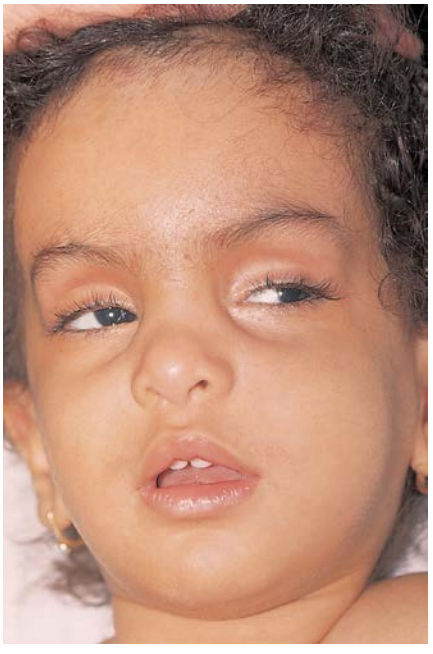

Figure 89.5 MO: The vacant facial expression. She had bilateral cherry red spots.

mitral regurgitation were observed in a 14-month-old who had marked overall regression at 18 months [9]. Computed tomography (CT) scan may reveal the Turkish moustache sign (Figure 89.12). Alimentation requires tube feeding. Death usually occurs between the ages of one and four years, most often from bronchopneumonia or aspiration.

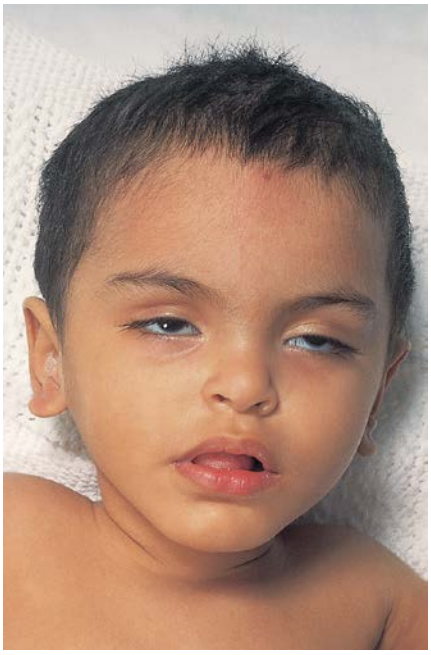

Figure 89.6 TO: Indicating the similar facial expression.

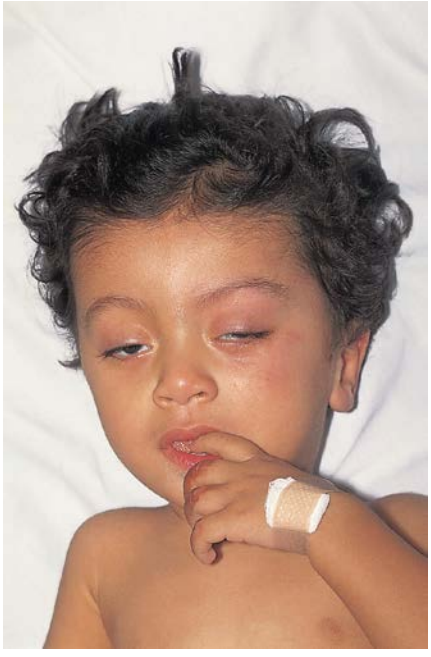

Figure 89.7 AMH: This 14-month-old boy with Sandhoff disease had a similar facial appearance, cherry red macular spots, acoustic myoclonus and no detectable leukocyte hexosaminidase activity.

At autopsy, the visceral organs may be somewhat heavier than those of patients with Tay-Sachs disease [7]. Visceral storage may be evident in lipid-laden histiocytes [2]. Some patients may have clinical hepatosplenomegaly, but most do not. Renal tubular cells show lipid deposits. Foamy histiocytes may be seen in bone marrow aspirates. In the brain, there is a typical neuronal lipidosis. Membranous

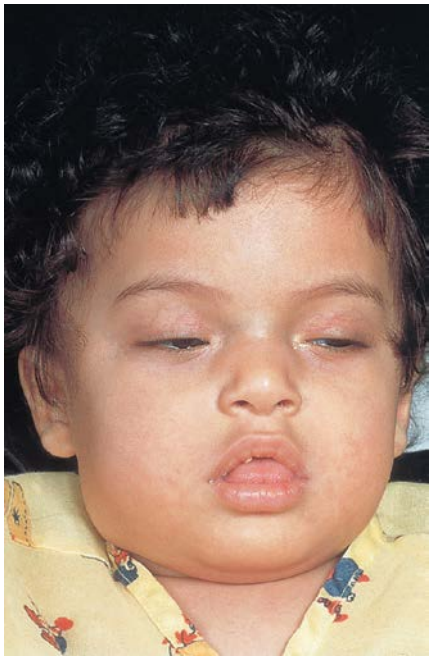

Figure 89.8 MSO: An 11-month-old with Sandhoff disease had a similar facial appearance and cherry red spots.

cytoplasmic bodies seen by electronmicroscopy are identical to those which are characteristic of Tay-Sachs disease. Vacuolated cells and lamellar inclusions are demonstrable by conjunctival biopsy.

A small number of variant later-onset forms of Sandhoff disease have been observed. They have variously been referred to as juvenile, subacute or adult-chronic, but now that there are molecular distinctions, it is likely that there will be a spectrum of phenotype. The majority of these variants present between two and ten years of age, most often with ataxia, speech abnormalities, or incoordination [10–15]. There may be choreoathetosis or dystonia. Neurodegeneration is progressive, and seizures and spasticity develop. There may or may not be cherry red spots or, more commonly, retinitis pigmentosa and optic atrophy. By 10–15 years, the patient is blind and decerebrate, as in the infantile patient, and death shortly ensues. One patient referred to as having a juvenile form of Sandhoff disease [11] developed slurred speech, ataxia and some mental deterioration at five years of age. By ten years, he had spasticity, but the optic fundi were normal.

Adult-onset patients may have psychiatric symptoms [14]. They have included confusion, emotional lability and intermittent psychosis. Decline in cognitive function was found in 44 percent of patients. Ataxia and an apparent spinocerebellar picture may be evident [10–15]. Dysarthria may be severe. The disorder may be very slowly progressive. Sensory neuropathy was a predominant finding in one family [15]. In late-onset patients, typical membranous cytoplasmic bodies have been reported in the myenteric plexus [16].

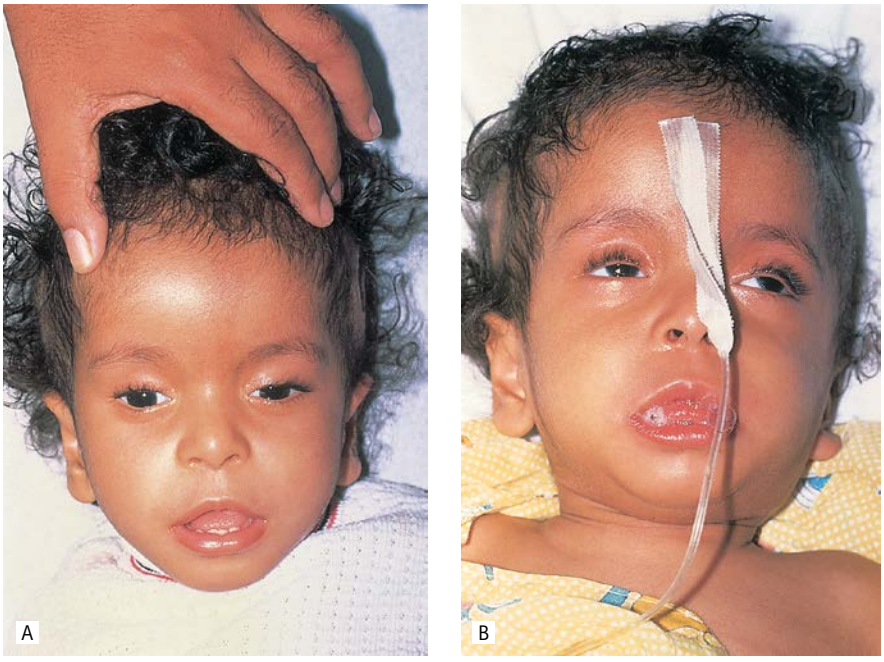

Figure 89.9 (A and B) A boy with Sandhoff disease at 18 and 19 months indicating the change in expression with progressive degeneration. He was blind and had cherry red spots at the time of the first photograph.

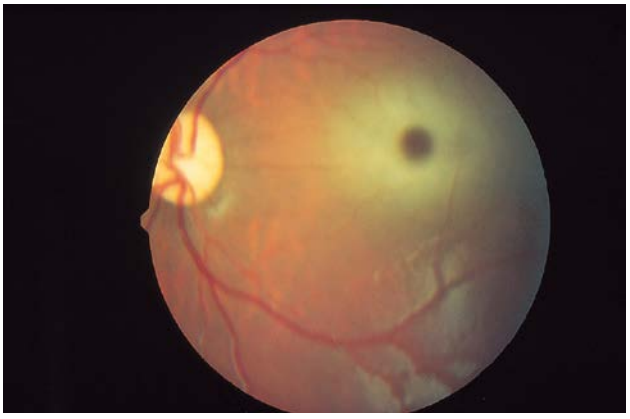

Figure 89.10 SO: The cherry red spot.

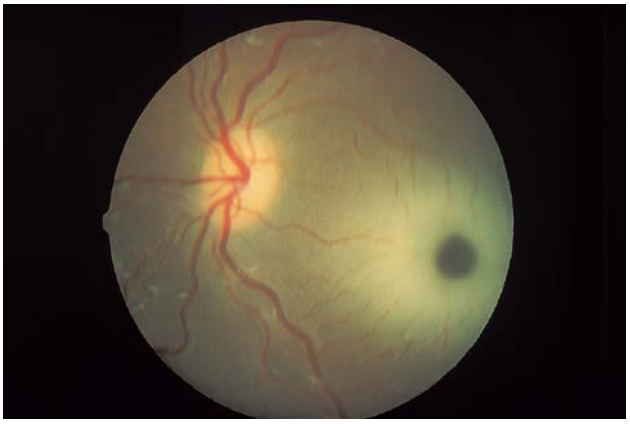

Figure 89.11 TO: The cherry red spot of an 18-month-old with Sandhoff disease. A cousin died at 17 months with a similar picture. Two brothers married two sisters. Hexosaminidase activity of both parents was in the heterozygote range.

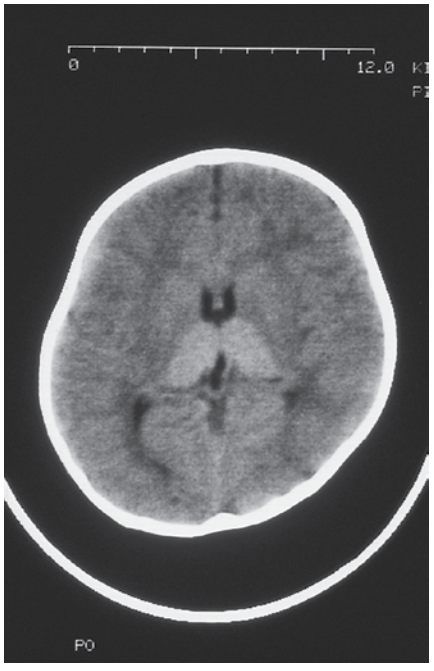

Figure 89.12 CT scan of the brain of a patient with Sandhoff disease indicating the Turkish moustache sign.

GENETICS AND PATHOGENESIS

Patients with Sandhoff disease accumulate GM_2 ganglioside in the brain [1]. The amounts found are 100–300 times the normal concentrations and quite similar to those of Tay-Sachs disease. In contrast to patients with Tay-Sachs disease, these patients also accumulate globoside, the common neutral glycolipid of erythrocyte and renal membranes,

which has the same amino terminal sugar as GM_2 ganglioside, N-acetyl galactosamine, in extraneural tissues, especially the liver, kidney, and spleen [17–20]. In the brain, there is storage of GM_2; in addition, the asialo derivative of GM_2 (GA_2) accumulates, and this too is a difference from Tay-Sachs disease. Globoside may be demonstrated in urinary sediments and plasma [18]. The stored compounds are all structurally related. The asialo derivative differs from GM_2 in the absence of the N-acetylneuraminic side chain, whereas globoside contains an extra galactose moiety. GA_2 is found in the brain in Sandhoff disease in amounts 100 times normal [17]. Oligosaccharides and glycopeptides, which have a glycosidically bound N-acetylhexosamine, accumulate in various tissues, and they are excreted in the urine [21, 22], providing a readily accessible approach to diagnosis.

Sandhoff disease is characterized by the lack not only of Hex-A, but also of Hex-B [7, 20–24]. The enzyme defect has been demonstrated with natural substrates, including GM_2, GA_2, and globoside, as well as with the artificial substrates nitrophenylacetylglucosaminide and 4-methylumbelliferyl-N-acetylglucosamine [1, 7]. The deficiency is present in all tissues of the body. It is readily assessed in serum, leukocytes, and cultured fibroblasts [17]. In the classic infantile patients, the activity of each enzyme is about 1–3 percent of normal [17]. Distinctions among the variant GM_2 gangliosidoses cannot be done well using the usual assays with artificial substrate, because there is a poor correlation between severity of clinical phenotype and degree of enzyme residual activity [25].

Each of the Hex-A and Hex-B enzymes has a molecular weight of about 100,000. The subunits are about 25,000 daltons. Both hexosaminidases cleave the lipids globoside and GA_2, but only Hex-A hydrolyzes GM_2. The β chain is coded for on chromosome 5, while the α chain is determined by a locus on chromosome 15. Cells from patients with Sandhoff disease lack the β chains [26]. In some examples of Sandhoff disease, the residual hexosaminidase in the liver has been shown to have an increased Km, and pH optimum, indicating that there is a structural gene alteration [23]. In somatic cell hybrids, there was independent segregation of Hex-A and Hex-B, consistent with their loci on two different chromosomes [27, 28]. Hybridization of fibroblasts from a patient with Tay-Sachs disease with those of a patient with Sandhoff disease revealed complementation in which hexosaminidase activity appeared, although it was present in neither parental strain [29]. Correction of Hex-A activity represented provision of the α subunit from the Sandhoff fibroblasts and the β unit from the Tay-Sachs cells to form a hybrid heteropolymer [29–31].

Sandhoff disease is transmitted as an autosomal recessive trait. The gene for the disease appears to be unusually prevalent in Lebanon [32]. It is also high in an area north of Cordoba in Argentina [33]. In Saudi Arabia [34], it is one of four frequently encountered lysosomal storage diseases. Unlike the others, Sandhoff disease was tribal in the sense that half of the patients were of one large tribe. In California, there is an increased frequency among Hispanic people of Mexican or Central American origin [35]. Most recent estimates [36] of carrier frequency have yielded a frequency of one in 278 for carriers of the Sandhoff gene in non-Jews and one in 500 in Jews. This would yield a frequency of infants born with the disease of one in 300,000 non-Jews and one in a million Jews. Newborn screening has been accomplished by analysis of enzyme activities in spots on filter paper [37].

The Hex-B gene contains 14 exons and spans approximately 45 kb [37, 38]. There is extensive homology with the Hex-A gene, as there is between the two α and β proteins. The mutations observed in Sandhoff disease have been heterogeneous [11, 37–39]. Some yield subunits that are cross-reacting material (CRM) positive; others are CRM negative. In the classic infantile Sandhoff disease, there have been a number of partial deletions; there may be normal or reduced amount of mRNA, but the activity of Hex-A is always essentially undetectable [5]. In a patient with later onset at five years [11], whose variant was referred to as hexosaminidase Paris [40–42], hexosaminidase B activity was deficient, but there was preservation of some activity of Hex-A. Another variant had considerable activity of both isozymes in serum but marked reduction in tissues [38]. In general, among the so-called juvenile variants, Hex-A activity has been expressed at 1–3 percent of control [43].

Mutations identified in classic infantile patients have usually been major alterations [44]. In addition to the 16 kb deletion involving the promoter and exons 1 to 5 [6], a 50 kb deletion was found in a single family [45]. At least one splicing mutation led to almost complete absence of mRNA.

Among later-onset patients, many were compound heterozygotes, such as I207V and Y456S [46]. In a family in which there was compound heterozygosity for P417L and the severe 16 kb deletion which, when homozygous, leads to the classic infantile disease, there was late onset presentation in the 51-year-old proband and four asymptomatic patients, 51–61 years of age [47]. In the hexosamidase Paris, the Hex-B minus, Hex-A plus phenotype, a duplication straddling intron 13 and exon 14 generated an alternative splice site and caused an in-frame insertion of 18 nucleotides for the mRNA. The second allele was not known. The 16 kb deletion has been observed in French and French-Canadian patients. Among the Argentine patients, there were two null mutations, a G-to-A transition (IVS 2 + 1) and a four-base deletion (784del4) [48]. Homozygosity for p.R284* was found in a girl from Jordan [8]. Among Italian patients [5] 11 different mutations were observed, 6 of them novel. A premature termination in the transcript of c.965delT led to degradation of the mRNA. A splice site mutation c.299+G>A in a patient with c.1538T>C was found in a patient with cardiac disease [9].

Detection of heterozygous carriers is possible by enzyme assay, which reveals amounts of Hex-A and Hex-B in leukocytes, skin, cultured fibroblasts, and serum that are

intermediate between normal and patient concentrations [49–53]. Heterozygotes have been reported [9] in whom the activity of the A isozyme was present, but the B isozyme was less than 20 percent of normal. In heterozygotes, the B isozyme was more thermolabile than normal [53], indicating the presence of a heteropolymer containing mutant and normal β chains. Intrauterine diagnosis has been accomplished by assay of cultured amniocytes [54–56]. The detection of N-acetylgalactosaminyloligosaccharides in amniotic fluid has been used for prenatal diagnosis, as it has in the urine for postnatal diagnosis [57, 58]. In a family in which the mutation is known, mutational analysis is the method of choice for prenatal diagnosis and for heterozygote detection.

TREATMENT

The treatment of Sandhoff disease is entirely supportive, but a variety of experimental approaches to therapy are being explored. An animal model for Sandhoff disease in cats and a knockout mouse permit rational studies of therapy [59–61]. These include enzyme replacement, bone marrow transplantation, and gene therapy. In the mouse, bone marrow transplantation prolonged lifespan from four or five months to eight months and appeared to slow neurologic degeneration, but there was no improvement in storage of glycolipid in brain or neuronal pathology [61]. Bone marrow transplantation in a patient with Sandhoff disease appeared to be without beneficial effect [62].

Substrate deprivation therapy in which an inhibitor lowers the synthesis of glycosphingolipid has been employed in the Sandhoff mouse, with N-butyldeoxynojirimycin (NB-DNJ), an inhibitor of glucosylceramide synthase [63]. Treatment prolonged lifespan and reduced storage of GM_2 and GA_2 in brain. It is expected that treatment would be of greater utility in later-onset phenotypes, rather than in the infantile form of the disease.

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90

Gaucher disease

MAJOR PHENOTYPIC EXPRESSION

Type 1: Splenomegaly, pancytopenia, hepatomegaly, bony pain, fractures, avascular necrosis.
Type 2: Acute neuronopathic: Early infantile onset, hypertonicity, seizures, trismus, retroflexion of the head; splenomegaly; hepatomegaly; rapid neurologic deterioration and death between one and 24 months.
Type 3: Subacute neuronopathic: Splenomegaly, hepatomegaly; childhood onset of neurologic manifestations – ataxia, spastic paraparesis, seizures, ophthalmoplegia; death in childhood or adulthood if untreated.
Lethal perinatal: collodion skin hepatosplenomegaly, hypotonia, hydrops fetalis.
All types: Accumulation glucocerebroside (glycosylceramide) and defective activity of lysosomal acid β-glucosidase; A mutation chromosome 1q922

INTRODUCTION

Gaucher disease is the most common of the lysosomal storage diseases. It was first described in 1882 by Gaucher [1], then a French medical student. He identified the pathognomonic cells, which are now known as Gaucher cells, in a 32-year-old woman with massive enlargement of the spleen. The eponym Gaucher disease was first employed by Brill in 1905 [2]. This phenotype, now referred to as type 1, was recognized in the 1950s to be common in Ashkenazi Jews [3]. Three other types of disease are known (Table 90.1). The acute neuronopathic early infantile, type 2, disease was described in 1927 [4, 5]. In 1959, a type 3, subacute neuronopathic disease was described in an isolated population in northern Sweden [6]; this slowly progressive neurologic disease is referred to as the Norrbottnian form, after the place of origin of the initial patients. Actually, each of the forms of the disease is panethnic.

The perinatal lethal form of the disease is now considered to be a distinct form of type 2. Actually, genetic heterogeneity is such that there are increasing numbers of intermediate and overlapping forms. The separate designations are useful because type 1 is so common and of course the neonatal lethal disease is very different.

Recognition of Gaucher disease as a reticuloendothelial storage disease was as early as 1907 [7] and, in 1924, the

Table 90.1 Clinical presentations of Gaucher disease

	Type 1	Type 2	Type 3	Lethal Neonatal
Onset	Infants child/adulthood	3–6 months	Childhood	Neonatal
Neurodegeneration	Absent	++++	++ → ++++	++++
Survival	6–80+ years	<2 Years	2nd to 4th decade	Fetal, neonatal
Splenomegaly	++++	++	++	++
Hepatomegaly	++	+	+	++
Fractures – bone crises	+	–	+	-
Ethnic predilection	Ashkenazi	Panethnic	Norrbottnian Swedish	Panethnic

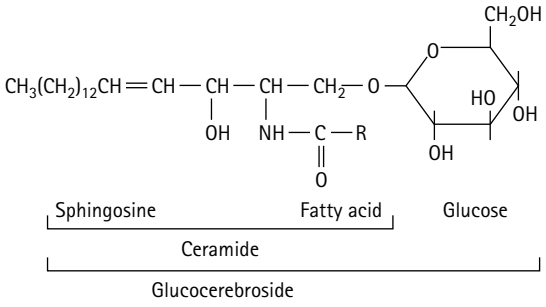

Figure 90.1 Structure of Glucocerebrosidase, the Gaucher lipid.

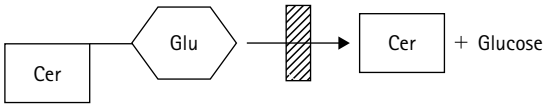

Figure 90.2 β-Glucocerebrosidase, the site of the defect in Gaucher disease.

stored material was identified as lipid and characterized as a cerebroside [8, 9]. Identification of the sugar in this cerebroside as glucose was reported by Aghion in 1934 in his thesis for the doctorate of philosophy (Figure 90.1) [10]. The molecular defect in glucocerebrosidase (Figure 90.2) was described in 1965, independently by Brady and colleagues [11], and by Patrick [12]. The defective enzyme is a lysosomal acid β-glucosidase, active in catalyzing the release of glucose from a number of substrates in addition to glucosylceramide. There is an activator of the enzyme, saposin C, which has a low molecular weight [13]. The gene for β-glucosidase is located on chromosome 1q21 [14]. The cDNA has been cloned and a number and variety of mutations have been identified [15–17]. The type 1 disease provides an interesting therapeutic model because enzyme replacement therapy has been quite successful [18]. Bone marrow transplantation may be curative.

CLINICAL ABNORMALITIES

Type 1

There is considerable heterogeneity of clinical expression. As many as 25 percent of affected individuals may be asymptomatic or have splenomegaly discovered incidental to an examination well into adult life, even into the eighth and ninth decades [19–22]. Severely affected individuals with type 1 disease may die in the first or second decade.

The initial clinical manifestation is usually painless splenomegaly [23, 24]. The spleen may be massively enlarged (Figures 90.3 and 90.4). It may be so large as to interfere with the intake of food into the stomach or to cause dispareunia. Splenic infarcts may occur. A large infarction may produce the picture of an acute abdomen, along with hyperuricemia. Radionuclide scans may be helpful in the diagnosis of the splenic infarcts. On the other hand, many patients are

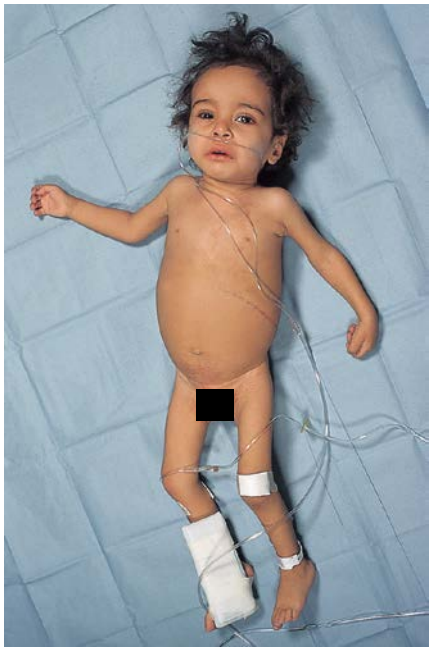

Figure 90.3 FTH: A two-year-old Saudi patient with Gaucher disease. Abdominal distention had been progressive and aossicated with weakness and failure to thrive. The liver was palpable 212 cm below the right costal margin. The spleen had been removed. ß-Glucosidase activity of fibroblasts was 9 mmol/mg per hour or 5 percent of normal.

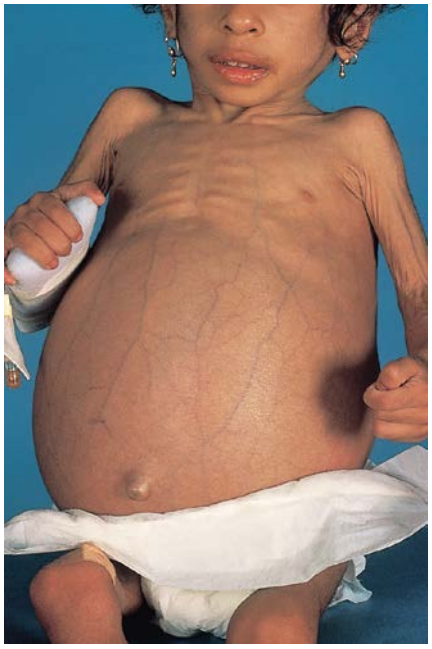

Figure 90.4 ZYAA: A three-year-old girl with Gaucher disease. The abdomen was enormous and the patient emacitated. The liver was palpated at 20 cm and the spleen at 17 cm below the costal margins. Hemoglobin was 6 g/dL and platelet count 45×103/mm^3. ß-Glucosidase activity was 1 mmol/hour per mg cells or 6 percent of normal.

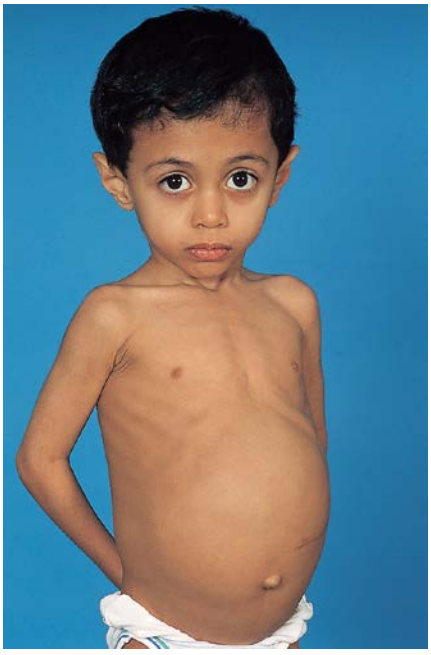

Figure 90.5 HFB: A four-year-old Saudi boy with Gaucher disease. The spleen had been removed, but the abdomen was distended by the enormous liver. In addition, he was thin, short, and wasted. Enzyme activity was 12 percent of control.

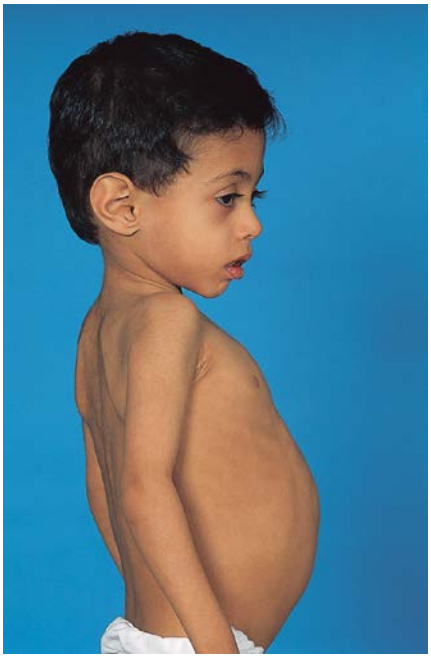

Figure 90.6 HFB: Lateral view highlights the abdominal distention. Parents were first cousins. IQ was 91. Treatment was initiated with ceredase (glucocerebrosidase).

estimated to have such mild disease that they never come to medical attention [19].

The liver is also enlarged (Figures 90.4–90.7), usually less than the spleen, but it may be as large or larger than the spleen (Figure 90.4) and it may be particularly prominent in

Figure 90.7 SSH: A three-year-old boy with Gaucher disease. The abdominal enlargement caused by the hepatomegaly is evident even with the patient fully clothed. β-Glucosidase activity was 5 percent of control. He had a splenectomy and recently had a successful bone marrow transplantation.

splenectomized patients (Figures 90.5–90.7). The large liver is usually not associated with liver disease, but there may be elevated transaminases, cirrhosis, esophageal varices, or hepatic failure [25–27]. Hepatic infarction may present as an acute abdominal catastrophe with a Budd-Chiari syndrome. One patient we saw was left with a large palpable notch in the center of the hepatic outline.

Thrombocytopenia is a common hematologic manifestation of Gaucher disease [26]. It may be accompanied by leukopenia and anemia, the full picture of hypersplenism, and resolves with splenectomy [28]. Late hematologic dysfunction in splenectomized patients may result from replacement of normal marrow with Gaucher cells. There may be bleeding, petechiae, and easy bruising.

Skeletal manifestations may be the chief or only complaint in some patients. Most patients have some bony abnormality. In many patients, they take the form of acute crises of pain, tenderness, redness, and swelling, mimicking acute osteomyelitis or the thrombotic crises of sickle cell disease.

The diagnosis of bony crisis is best made by technetium radionuclide scan [29]. There may be fever, leukocytosis, and elevation of the erythrocyte sedimentation rate. Pyogenic osteomyelitis is rare in Gaucher disease and usually follows some invasive procedure on bone; therefore, surgical diagnostic procedures are not recommended for crises [30].

Areas of focal destruction of bone, osteonecrosis, or avascular necrosis occur in the absence of acute crises, especially in the area of the femoral head [31]. A child with hip pain may be thought to have Legg-Calve-Perthes

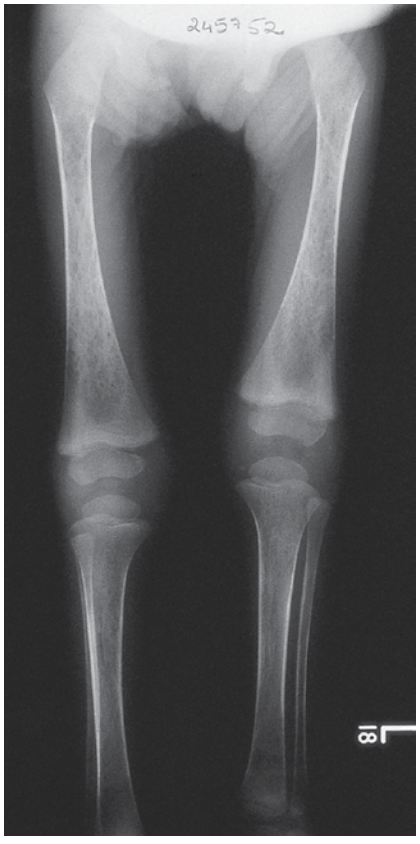

Figure 90.8 Roentgenogram of the lower extremities of a patient with Gaucher disease illustrates the osteoporosis and enlargement of the width particularly of the femur leading to an Erlenmeyer flask appearance.

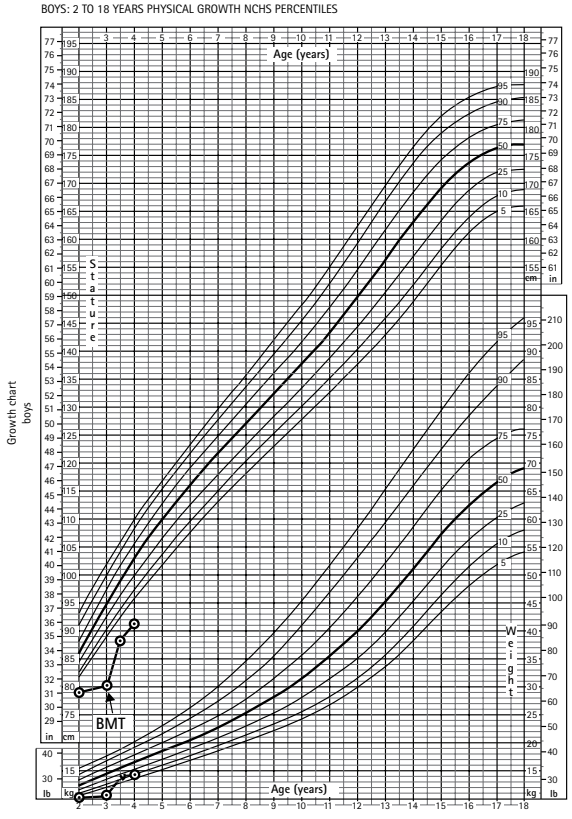

Figure 90.9 SSH: Acceleration in growth in height and weight following bone marrow transplantation. (Adapted with permission from Hamill PV, Drizd TA, Johnson CL et al. Physical growth: National Center for Health Statistics percentiles. *Am J Clin Nutr* 1979; 32: 607–29.)

disease [32]. Pathologic fracture is common. Some degree of osteoporosis is the rule in this disease. Compression fracture of vertebral bodies is a common complication [31, 33–35], and there may be radicular or spinal cord compression or kyphoscoliotic deformity. Roentgenograms reveal osteoporosis of the spine and compression fractures [36]. Magnetic resonance imaging (MRI) is more effective than conventional radiography or computed tomography (CT) scanning in evaluating the spine and effects on the cord and also in assessing areas of avascular necrosis [37]. The most common skeletal feature of Gaucher disease is the loss of modeling and increased width that leads to the Erlenmeyer flask appearance of the femur (Figure 90.8) [38]. In addition, areas of severe loss of bone density may alternate with areas of osteosclerosis and focal infarctions.

Growth and development may be altered drastically in Gaucher disease (Figure 90.9). Pubertal development may also be delayed. In addition to the effects of anemia, splenic enlargement, and chronic disease, resting energy expenditure is increased about 44 percent [39].

Pulmonary infiltration may occur less commonly (Figure 90.10) but may lead to pulmonary failure [40]. There may also be right to left intrapulmonary shunting of blood. Fingers may be clubbed. The skin may show yellow or brown pigmentation and a propensity to tan [41]. Patients

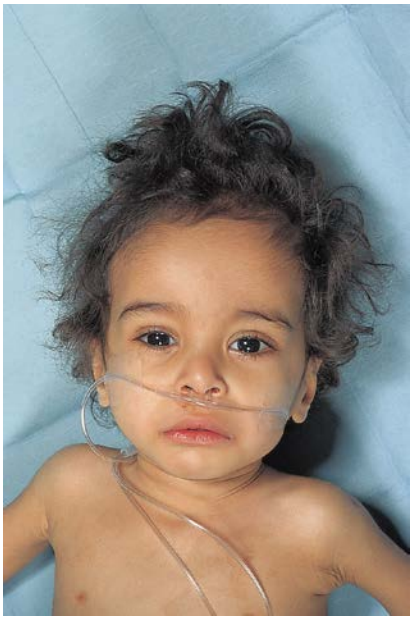

Figure 90.10 FTH: Involvement of the lung was documented by biopsy. The patient was oxygen-dependent. Roentgenogram revealed virtually completely opaque lung fields with air bronchograms.

with Gaucher disease appear to be susceptible to cancer, especially lymphoproliferative diseases [42], but there is considerable controversy as to whether there is statistically increased risk [43]. The nervous system is by definition spared in type 1 disease, but a number of secondary complications do affect the nervous system in addition to the complications of vertebral disease. These include cerebral fat emboli secondary to skeletal disease, neuropathy and hematomyelia secondary to bleeding [42]; a patient was reported with lumbosacral cauda equina syndrome secondary to an intrathecal sacral cyst, apparently the result of subdural hemorrhage [44].

A few patients with type 1 Gaucher disease have been found to develop parkinsonian symptoms [45], with onset typically in the fourth decade and a limited responsiveness to therapy. There has not been any association with genotype, but in several cases, there was a family history of parkinsonism [46]. In a study of tissue from patients with parkinsonism, alterations in glucocerebrosidase were found in 12 out of 57 individuals [47]. The association between parkinsonism and Gaucher disease remains to be explained. Malignancies including multiple myeloma and leukemia and monoclonal gammopathies have been reported in Gaucher disease [48, 49]. Extraosseous accumulation of Gaucher cells have been called Gaucheromas [49].

Type 2

Infants with acute neuronopathic Gaucher disease appear normal at birth, though splenomegaly may be found in the first three months, and they usually develop some early milestones. Early evidence of neurologic disease may be unusual irritability, a lack of alertness, apparent weakness in holding up the head, oculomotor apraxia, or a fixed strabismus [24, 50]. Neurodegenerative disease appears in six months and proceeds rapidly to a classic picture of spasticity and opisthotonus, with trismus, strabismus, and hyperextension of the neck [50]. There may be seizures, bulbar signs, or involuntary choreoathetoid movements. Visual fixation may be absent, as well as visual evoked response (VER). Atrophy may be evident on the MRI of the brain. Death usually results from apnea, aspiration pneumonia, or respiratory failure at an average age of 18 months. Autopsy reveals neuronal degeneration and neuronophagia.

The perinatal lethal disease has often been considered a subtype of infants with type 2 Gaucher disease in infants with a rapid fulminant neonatal onset course [50]. Death may occur as early as two months of age. This disorder is reminiscent of the disease in mice homozygous for a null glucocerebrosidase allele who die within 24 hours [51]. Infants with Gaucher disease may present with lamellar ichthyosis, which may take the form of the collodion baby [52, 53]. Presentation of Gaucher disease with non-immune hydrops fetalis appears to represent the same process [54]. Perinatal-lethal Gaucher disease with hydrops, ichthyosis, and fetal akinesia sequence has been associated with particularly severe mutations [55].

Type 3

In the classic Norrbottnian form of disease [56, 57], the patient's early manifestations may lead to a diagnosis of type 1 Gaucher disease. The neurologic keynote finding is myoclonus of cerebral origin [58, 59]. Multifocal jerky movements are widespread in the muscles and occur at rest or with movement. The electroencephalogram (EEG) may reveal spike discharges [60]. With time, there are generalized grand mal seizures [61, 62]. Careful examination of the eyes discloses deficits in saccadic velocities, progressive with time to paralysis of lateral gaze [63, 64]. There may be slow upward looping of the eyes [65]. As the disease progresses, ataxia and spasticity appear. There may be dementia.

A group of patients in whom paralysis of horizontal supranuclear gaze is the major neurologic sign have been considered as a subgroup of type 3 [48, 64]. They may have mild cognitive impairment. These patients often have aggressive systemic manifestations. Some authors have subdivided type 3 patients into groups, for example, designating those with progressive myoclonic epilepsy and dementia, and a poorer prognosis, as type 3a [64], but there are clearly unidentified genetic modifiers which determine prognosis [66]. When untreated, death in type 3 patients characteristically occurs in childhood or adolescence from pulmonary or hepatic disease and in those with progressive myoclonic epilepsy, neurologic deterioration may cause death in adulthood despite treatment. It appears that neuropathic Gaucher disease is a continuum [67] from severe neonatal phenotypes to adults with oculomotor problems.

Diagnosis

The diagnosis is often first made clinically by the recognition of Gaucher cells (Figures 90.11 and 90.12) in a bone marrow aspirate or biopsied tissue. These cells are large lipid-laden macrophages with foamy cytoplasm. The fibrillary pattern is quite different from that of Niemann-Pick cells. Electron microscopy reveals tubular structures. These cells are widely dispersed in tissues. The diagnosis may be suspected by the presence of elevated activity of acid phosphatase in plasma [68], a high level of ferritin [69], angiotensin-converting enzyme [70], and particularly chitotriosidase, which is induced in activated macrophages, and is very significantly elevated in most cases of Gaucher disease [70].

Definitive diagnosis requires the assay of acid β-glucosidase. This can be done in leukocytes or cultured fibroblasts [71–73]. Enzyme assay is not useful in distinguishing the various types of Gaucher disease.

Testing for the mutation in the DNA is useful not only in affected individuals but in testing for carrier status. It is usually done on leucocytes or cultured fibroblasts.

Testing for activity of the enzyme is still required for patients.

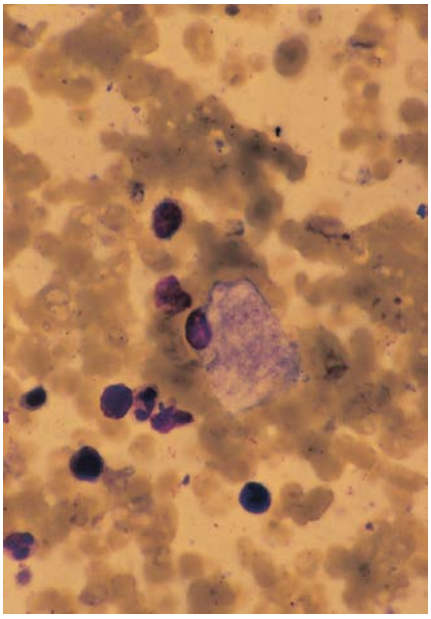

Figure 90.11 Gaucher cells. At this magnification, the size relative to leukocytes and erythrocytes is evident.

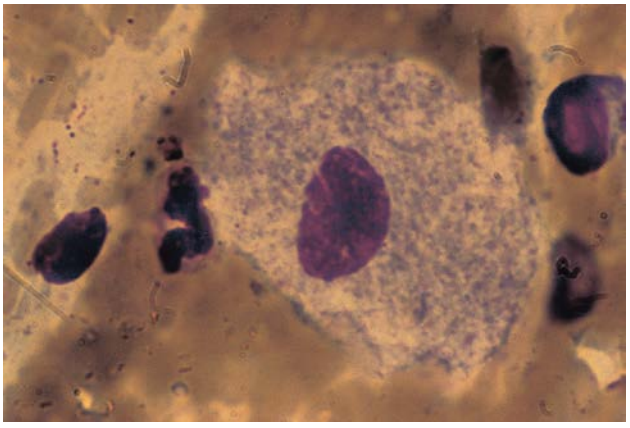

Figure 90.12 Gaucher cells illustrating the foamy cytoplasm.

GENETICS AND PATHOGENESIS

Gaucher disease is inherited in an autosomal recessive fashion. Most patients are of type 1, and this is most common in Ashkenazi Jews, in whom the incidence has been estimated at between one in 640 [24] and one in 855 [74]. This is the most prevalent genetic disorder in that population.

The gene for acid β-glucosidase has been cloned and linked to chromosome 1q21 [14, 17, 75]. It contains 11 exons and is approximately 7 kb in length. The cDNA is about 2.5 b [16, 76]. More than 300 mutations have been defined in patients with Gaucher disease [19, 77, 78]. Four are common enough to account for over 96 percent of the Ashkenazi Jewish patients (Table 90.2) [79, 80]. The A-to-G transition at position 1226 which causes an asparagine to serine substitution at amino acid 370 (N370S) [80] is the major cause of the disease in this population. The next most frequent is the frameshift mutation at 84GG [81], a mutation which leads to no enzyme protein. The point mutation which changes a leucine to proline at 444 (L444P) and the splice junction mutation at IVS2(+1)G>A account for most of the rest of mutations in this population [17, 28]. Individuals with the N370S mutation are diagnosed decades later than those with the other mutations [19]; and at least half of those with the N370S/N370S genotype are estimated never to come to medical attention [19].

Heterozygote detection is sometimes possible by enzyme analysis, but it is not reliable because of overlap with the normal population. In Jewish populations, heterozygote detection may be carried out by genotyping. This is less useful in non-Jewish populations; about 25 percent of these patients carry the N370S mutation and 35 percent the L444P mutation, and the rest are unidentified or rare.

Some correlations of phenotype with genotype have emerged from increasing information on the nature of mutation in Gaucher disease. The N370S mutation appears relatively conservative, and a majority of patients who carry this mutation have relatively mild disease. Homozygosity for this mutation excludes neuronopathic disease, but even a single 370 allele in a compound leads to an absence of neurologic disease [76]; compound heterozygotes tend to have more severe somatic disease than N370S homozygotes. The Norrbottnian population of Gaucher patients in northern Sweden are homozygous for the L444P mutation and have type 3 disease of variable severity [82]. The L444P mutation is also found in other populations, and though it is associated with severe somatic symptoms and generally with neurologic manifestations, in some patients neuronopathic disease may be absent [64]. The 84GG frameshift leads to severe disease and no enzyme activity, as does the IVS2(+1) G>A mutation in intron 2 [28, 79, 83]. The diagnosis is best confirmed by technetium radionuclide scan [29].

An index of complexity, and the power of molecular techniques to unravel it, is a family [84] in which two

Table 90.2 Common mutations in Gaucher disease

cDNA No.	Amino acid No.	Nucleotide substitution	Amino acid substitution	Type of mutation
1226	370	A → G	Asn Ser	Point mutation
84		G → GG		Frameshift insertion
1448	444	T → C	Leu Pro	Point mutation
IVS2 + 1		G → A		Splice junction mutation

children died of type 2 disease, and a son had relatively indolent type 1 disease. The mother and son had similarly low levels of β-glucosidase, while the father's level was consistent with heterozygosity. Molecular analysis revealed the mother, asymptomatic at 62 years, and the son to be S370N/L444P compounds. The father was heterozygous L444P/normal. It was assumed that the infants who died by one year of age had inherited two L444P alleles.

The consequence of defective activity of acid β-glucosidase is the accumulation of glycosylceramide (Figure 90.1). In ceramide, there is a long-chain fatty acid amide linkage at the carbon 2 of sphingosine. Glycosphingolipids with longer oligosaccharide moieties are successively degraded to glycosylceramide. The amounts of this compound stored in Gaucher disease are enormous. Deacylated glycosylsphingosine has also been found in tissues of patients [85]. It is thought that the accumulated compounds are toxic to certain tissues.

TREATMENT

Among the earliest approaches to provide a source of active enzyme in Gaucher disease was the use of bone marrow transplantation (Figure 90.9) [86]. The procedure may be essentially curative in type 1 disease, but there is a risk of mortality from the procedure. It certainly raises the possibility that gene therapy in which the normal gene is introduced into the patient's hematopoietic cells will one day be an option.

Meanwhile, enzyme replacement therapy has become a major advance in the management of this disease. It has revolutionized prognosis and has become the standard of care [87].

Gaucher disease was the first lysosomal storage disease for which this approach became available. The major breakthrough in permitting successful therapy was the recognition that lipid-laden macrophages have a mannose receptor [87]; modifying the glycoprotein glucocerebrosidase to expose a terminal mannose permits the enzyme to attach to and be incorporated into the macrophage [48]. A modified form of the enzyme purified from human placenta was approved for treatment in 1991 under the name Ceredase (algucerase), and then in 1994, a form of human glucocerebrosidase produced in cultured Chinese hamster ovary cells was approved under the name Cerezyme (imiglucerase). This has largely replaced alglucerase. Another form, produced in human fibroblasts, was approved in 2010 under the name VPRIV (velaglucerase).

Clinical responses to enzyme replacement therapy have been clearly evident in hematologic (anemia and thrombocytopenia), visceral, and even (with longer courses of treatment) bony disease. Decrease in organ size is evident within six months. Bone pains and crises diminish or disappear within 12–24 months [19], and, at least in children, roentgenograms of the bones have improved after years of treatment. Growth and development regularly improve. The treatment is expensive. There is still some controversy as to dose. The dose generally employed is 60 U/kg every two weeks [48], but 30 U/kg every two weeks may also be effective. Experience with 26 years of the use with imiglucerase [88] has led to excellent safety and efficiency. Dosage varied from 15 to 120 U/kg every other week, but the usual dosage was 60 U/kg. Enzyme replacement therapy has been judged [89] ineffective in intraalveolar pulmonary disease.

There is logic in using small frequent infusions considering that the macrophage receptors constitute a low-capacity, high-affinity system, but practical considerations of intravenous (IV) access and demands on the patient's time have made the larger, less frequent dosing much more popular. It is possible that maintenance requirements for enzyme will be lower after the initial removal of accumulated glycolipid [90], but there is a risk of relapse in some patients. The expense of treatment ($100,000 to $200,000 per year) requires judicious use [19].

Another approach, called substrate reduction therapy, is possible with an inhibitor of ceramide glucosyltransferase, and the agent N-butyldeoxynojirimycin was approved in 2004 (miglustat) for adult patients with Gaucher disease for whom IV enzyme replacement is not practical [90–92]. Results are similar to those [93] with enzyme therapy [19]. Studies are underway using a more specific glucosylceramide synthase inhibitor, eliglustat tartrate [94].

An alternative approach is to use pharmacologic chaperones to stabilize products of missense mutations, and there are efforts to develop such agents, including isofagomine [95]. The c.1226A>G (N370S) mutation, which is found in >98 percent of Jewish patients and about half of non-Jewish patients, is effectively treated with the chaperones N-(n-nonyl) deoxynojirimycin [91]. Activity in homozygous cells *in vitro* was increased two-fold in concentration of 10 μM.

It is generally agreed that enzyme replacement is not effective in type 2 disease, while in the type 3 disease, systemic improvement is accompanied by no change in cerebral manifestations [96]. At present, only supportive measures are available for type 2 disease. Piracetam may be helpful in the management of myoclonus [97].

A variety of modalities are used to monitor the progress of therapy in Gaucher disease. Dual-energy X-ray absorptiometry (DXA) is sensitive to changes in bone mineral density and generalized osteopenia, but is insensitive to local changes and cannot reliably predict fracture risk [98]. It has been recommended that conventional MRI be used, and methods using chemical shift reflect the differences in the resonant frequencies of fat and water in bone marrow and can, therefore, detect the reduction in the fat fraction of bone marrow that occurs with infiltration in Gaucher disease. Preferred method is quantitative chemical shift imaging [99], but that is not widely available. Other approaches for semi-quantitative estimation of bone marrow burden of disease have used a scoring system [100]. The most sensitive biomarker that correlates with the disease activity is chitotriosidase [69], but approximately 6 percent of the general population has

no activity, resulting from a panethnic inactivating 24-bp duplication [101]. Another marker, CCL18 (also known as PARC/MIP-4/DC-CK1), is elevated 10- to 50-fold in symptomatic Gaucher disease patients [102].

A variety of supportive measures may be rendered unnecessary by the early use of enzyme replacement. There is, in general, no longer a place for splenectomy, but it might be considered in a patient with extensive thrombocytopenia or cardiopulmonary symptoms from a massive spleen. Hip replacement is the preferred modality for avascular necrosis. Replacement of other joints may be necessary. The avoidance of injury to bone, especially in sports is prudent. The frequency and severity of crises of bone pain and fractures were reported to improve in patients treated with biphosphonates [103], which are analogs of pyrophosphate that bind to hydroxyapatite, inhibiting resorption. A placebo-controlled trial of alendronate showed significant benefit as an adjuvant to enzyme replacement, with improvement in bone density and bone mineral content within 18 months [104].

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Niemann-Pick disease

MAJOR PHENOTYPIC EXPRESSION

Type A: Hepatosplenomegaly, neurologic degeneration, failure to thrive, cherry red macular spot, foam cells in bone marrow, storage of sphingomyelin and deficiency of lysosomal acid sphingomyelinase.
Type B: Hepatosplenomegaly, pulmonary infiltration, foam cells, storage of sphingomyelin and deficiency of sphingomyelinase.

INTRODUCTION

The disease was first described by Niemann in 1914 [1] in an infant with hepatosplenomegaly who died at 18 months after progressive neurologic deterioration and was found to have large foam cells in the liver and spleen. Pick's contribution [2, 3] was to distinguish this disorder from Gaucher disease on the basis of the appearance of the foam cells. Phenotypic variation became apparent with additional reports [4, 5], especially of adults with hepatosplenomegaly, but no neurologic abnormality in what has come to be called type B [6]. In 1934, the stored lipid was identified by Klenk [7] as sphingomyelin (Figure 91.1). In 1966, Brady *et al.* [8] identified the deficiency of sphingomyelinase (EC 3.1.4.12) (Figure 91.2) as the cause of Niemann-Pick disease. The deficiency was also readily documented in a type B patient [9].

The discovery of the enzyme permitted the categorization of other patients classified as Niemann-Pick disease who clearly did not have sphingomyelinase deficiency as their molecular etiology, such as type C (Chapter 92). The separation of type C is an important distinction, but now that the nature of mutation has begun to be defined, separation into types A and B begins to appear artificial. There are certainly some very distinct phenotypes among the deficiencies of sphingomyelinase; soon the genotype for each will be known. It is already clear that there are quite distinct genotypes. Type A disease is relatively rare except in Ashkenazi Jews [5, 10, 11], in whom type B is quite rare. Various phenotypes of type B are found

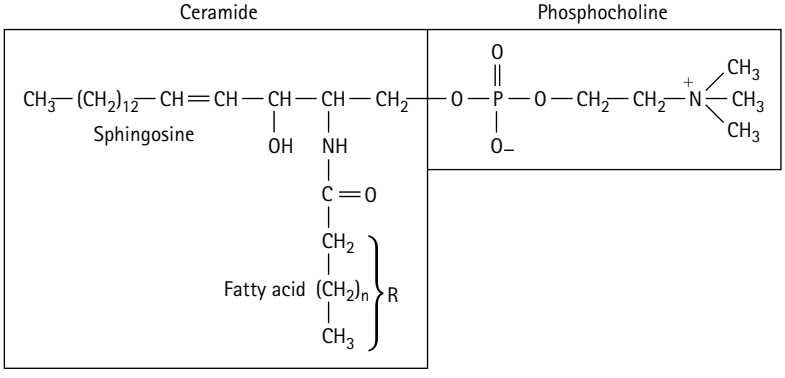

Figure 91.1 The structure of sphingomyelin.

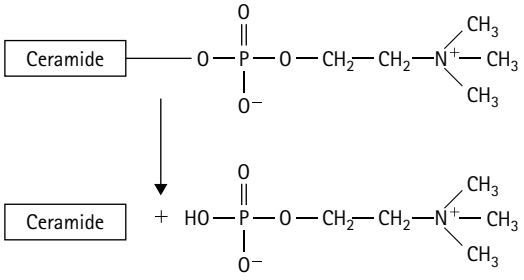

Figure 91.2 The sphingomyelinase reaction, the site of defect in Niemann-Pick disease.

commonly in Arabs, Turks, and Portuguese [12]. Somatic cell hybridization indicated clearly that types A and B represented allelic variation in a single gene [13] and that type C was different. Molecular studies were confirmatory.

The cDNA for sphingomyelinase has been cloned and sequenced [14, 15]. The gene has been mapped to chromosome 11 p15.1-p15.4 [16]. A number of mutations have been identified in both type A and type B patients [12, 17, 18]. Distinct mutations have been found in the ethnic groups in which Niemann-Pick disease is common.

CLINICAL ABNORMALITIES

Type A

The acute infantile form of Niemann-Pick disease usually presents first with massive enlargement of the liver and spleen [22] (Figures 91.3, 91.4, and 91.5) [5, 6, 10, 19–21].

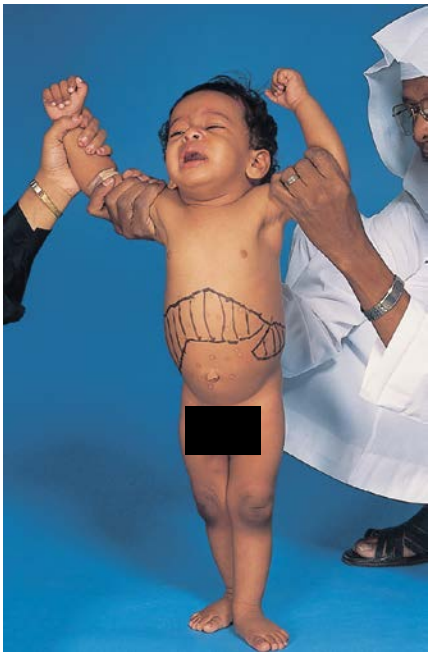

Figure 91.3 A patient with infantile Niemann-Pick disease. The hepatosplenomegaly is outlined.

Some patients have neonatal edema and hydrops fetalis may occur [23]. The liver and spleen may be enlarged at birth, and storage of lipid has been documented in the liver, brain, kidney, and placenta prior to birth [19, 23, 24]. Placental enlargement has been shown by ultrasound [25], and it is thought that storage in the placenta may lead to fetal loss [19]. The abdomen is protuberant. The liver seems always

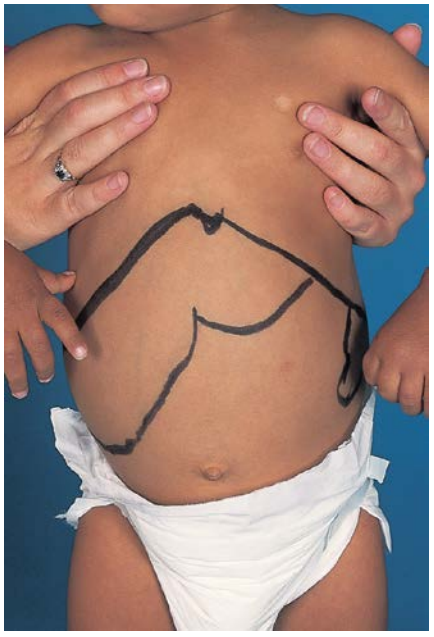

Figure 91.4 Another patient with massive hepatosplenomegaly resulting from Niemann-Pick disease.

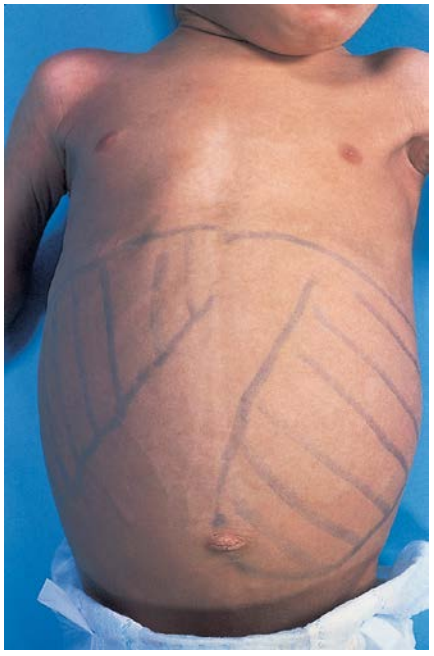

Figure 91.5 Massive hepatosplenomegaly in an 11-month-old infant with Niemann-Pick disease. Abdominal distension was noted at birth. He had jaundice and acholic stools at six months, and repeated pulmonary infections.

to be enlarged out of proportion to the spleen, in contrast to Gaucher disease (Chapter 90).

We have seen Niemann-Pick patients with hepatosplenomegaly whose history was that the spleen was not palpable early. Transaminases aspartate transaminase (AST) and alanine transaminase (ALT) are elevated, at least at times [26]. The alkaline phosphatase is also usually elevated. The cholesterol may be elevated in addition. There may be prolonged neonatal jaundice, and episodes of unexplained jaundice later. We have seen patients who presented in early infancy with acute jaundice, abnormal liver function tests, and hepatomegaly, suggesting a diagnosis of acute hepatocellular disease rather than a lipid storage disease. We have also seen a patient in whom two liver biopsies had been done in another institution and interpreted as fatty metamorphosis. At least one patient with Niemann-Pick disease was thought, on biopsy, to have glycogenosis [27]. Jaundice is a common terminal finding and some patients have developed disseminated intravascular coagulopathy. There may be lymphadenopathy.

By six months, episodes of respiratory distress occur, which may require oxygen. Some episodes are clear-cut infections, such as bronchiolitis or pneumonia but, in others, infection is not obvious. Some patients have had noisy respirations and rhinorrhea from birth [28]. Chest film may reveal diffuse interstitial infiltrates in a reticular or finely nodular pattern [29]. Patients may also have unexplained fevers.

Failure to thrive is evident by eight to nine months of age. Weight gain stops, but increase in linear growth may not stop until 15–18 months of age, and so the patient looks increasingly cachectic. Anorexia may be complicated by vomiting, and there may be some diarrhea [30, 31] or constipation.

Neurologic involvement may be first evidenced in a failure to achieve milestones, such as sitting, but some have developed normally for six months [28], or as long as one year [6]. Progression of disease occurs with loss of milestones achieved. Patients may appear weak or hypotonic. Deep tendon reflexes are exaggerated. Neurologic degeneration is progressive to a rigid state with spasticity in which there appears to be no consciousness of the environment. Seizures are not common; the electroencephalograph (EEG) is usually normal. Cherry red or cherry black (dependent on the pigment of the patient) macular spots (Figure 91.6) are seen in about 50 percent of the patients. In one series of patients, [19] all patients had cherry red spots by one year of age. Sometimes, there is a sprinkled salt appearance around the macula, a gray granular appearance, the macular halo syndrome, or melting snow appearance [32–35]. The electroretinogram is abnormal.

Brownish-yellow discoloration may develop in the skin [36]. Xanthomas have been described, particularly on the face and arms [5, 10, 11]. Most patients develop osteoporosis. A hypochromic, microcytic anemia may be followed with time by thrombocytopenia or granulocytopenia. The terminal episode may be with asphyxia or pneumonia.

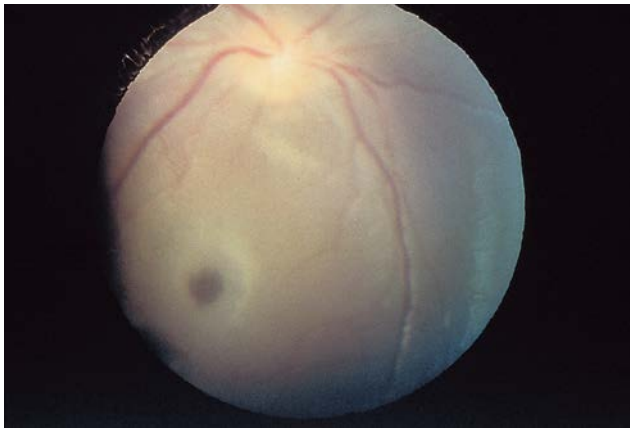

Figure 91.6 The cherry red macular degeneration in a ten-month-old child with Niemann-Pick disease.

Type B

Type B patients represent quite a varied spectrum, from those diagnosed in infancy because of hepatomegaly or hepatosplenomegaly to those first detected in adulthood, as expected for a variety of different mutations. Nevertheless, the type A phenotype is much more common and accounts for about three-fourths of all patients. We suspect that among the type B patients, there are a number of distinct phenotypes that are beginning to correlate with genotype.

What we think of as the classic type B patient is an adult or older adolescent who comes to attention because of splenomegaly found incidentally on physical examination (Figures 91.7 and 91.8) [37–39]. Some of these patients have had sea-blue histiocytes in marrow [37] or tissues [39] and this type has been called the Lewis variant [37]. Such patients may have elevation of the serum level of acid phosphatase. The King Faisal series included 35 patients. Pancytopenia may result from hypersplenism, and splenectomy may be required. Splenic rupture has been described [37]. Patients have been described in whom there were no neurologic abnormalities well into adult life [5, 39–41]. This may be one phenotype.

Others with a relatively mild phenotype may have some neurologic features. Extrapyramidal signs were reported in one family [42]. Impaired mental development was reported in unrelated patients at nine and 18 years [43]. A number of patients has been reported with cerebellar ataxia [18, 42, 43]. Some of these may have been patients who had Niemann-Pick type C disease. Patients have had cherry red spots or other grayish macular pigmentation about the macula, often with no other neurologic manifestation [32, 33, 39, 44–47]. Evidence of abnormal neural storage has been observed despite absence of neurologic abnormalities [48]. Two sisters without impaired mental development had inclusion bodies in exons and Schwann cells of rectal biopsies, and vacuolated macrophages in the cerebrospinal fluid (CSF) [48].

Figure 91.7 A 39-year-old man with Lewis variant of Niemann-Pick disease. The spleen was palpable 6 cm below the costal margin. The liver was at 4 cm. Sphingomyelinase activity of fibroblasts was 18 percent of control.

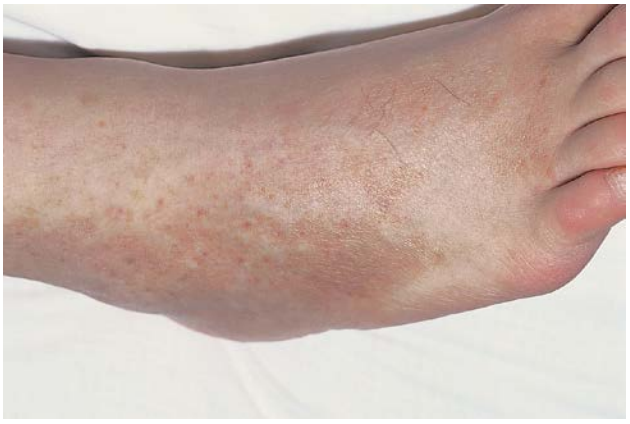

Figure 91.8 Brownish pigment on the dorsal and lateral ankle correlated with a loss of vibratory sensation in the area.

Some others that have been included in type B have had quite severe, and early-onset disease. We think of this as the Saudi variant [49, 50]. Early symptoms are failure to thrive and abdominal distension. On examination, the spleen is huge (Figures 91.3–91.5). The liver may be just as huge or even more so. They have been said not to have neurodegenerative disease, but they all have cherry red macular spots (Figure 91.6). Furthermore, two patients who survived infancy and bone marrow transplantation went on to develop white matter changes in the central nervous system (CNS) and neurologic manifestations. The facial appearance may develop similarities (Figure 91.9).

Patients are hypotonic and developmentally delayed. One patient at 20 months could sit, but could not crawl or stand [50]. She could speak two or three words. Cachexia is prominent early (Figures 91.10 and 91.11). Most of these patients die by three years of age. Terminal events include bleeding, anemia, and thrombocytopenia, often requiring daily transfusion of platelets, and hepatic failure. In a series of Saudi patients, some of whose pictures are shown in this chapter, five died between 18 and 36 months; one survivor to 4.5 years had had a bone marrow transplantation. Pulmonary infiltration is evident in roentgenograms as miliary nodular lesions [50]. Pulmonary function may be abnormal, and there may be complicating pneumonia. Liver function tests, alanine aminotransferase, and aspartate aminotransferase may be elevated, along with triglycerides. Abdominal ultrasound documents the hepatosplenomegaly.

Other patients may have hepatic or pulmonary disease. Liver disease, either biliary cirrhosis [51] or cirrhosis, may be life-threatening, and portal hypertension and ascites may develop [52]. This latter picture was reported in an otherwise adult-type disease [52]. Pulmonary disease has also been reported in adult-type disease [53]. In addition to the diffuse infiltration seen on roentgenograms, there may be exertional dyspnea and decreased pO_2 because of diminished diffusion. Bronchopneumonia may develop, and/or cor pulmonale [53].

In a series of type B patients in the United States, in whom sphingomyelinase deficiency and the mutation were documented, height and weight were usually low; 39 percent and 21 percent were below the fifth percentile for height and weight, and these correlated with large organ volumes [54]. Bone age was also behind 2.5 years.

COMMON PATHOLOGIC FEATURES

The pathognomonic feature of all patients with deficiency of sphingomyelinase is the foam cell (Figure 91.12). This large (20–90 μ) cell or macrophage is most commonly first detected in bone marrow aspirate. As a reticuloendothelial cell, it is found widely in the spleen, liver, lymph nodes, and lungs. In stained preparations, the cells have a foamy appearance that results from the stored material, which stains positively with stains for lipids. The lipid droplets are uniform in size, and the appearance has been called honeycomb-like or mulberry-like. The cytoplasm of these cells stains blue with Wright stain, which gives rise to the sea-blue histiocyte designation [37]. It is clear now that sea-blue histiocytes, once thought to represent a distinct disease [55, 56] are seen in sphingomyelinase deficiency [39]. On electron microscopy (Figures 91.13 and 91.14), foam cells have small eccentric nuclei and membrane-bound lucent areas from which storage material has been dissolved. There may be granular material, whorls, or lamellae. There may be infiltration in the gastrointestinal tract, which might account for intestinal symptoms and failure to thrive, and diagnosis has been made by rectal biopsy. Storage is seen

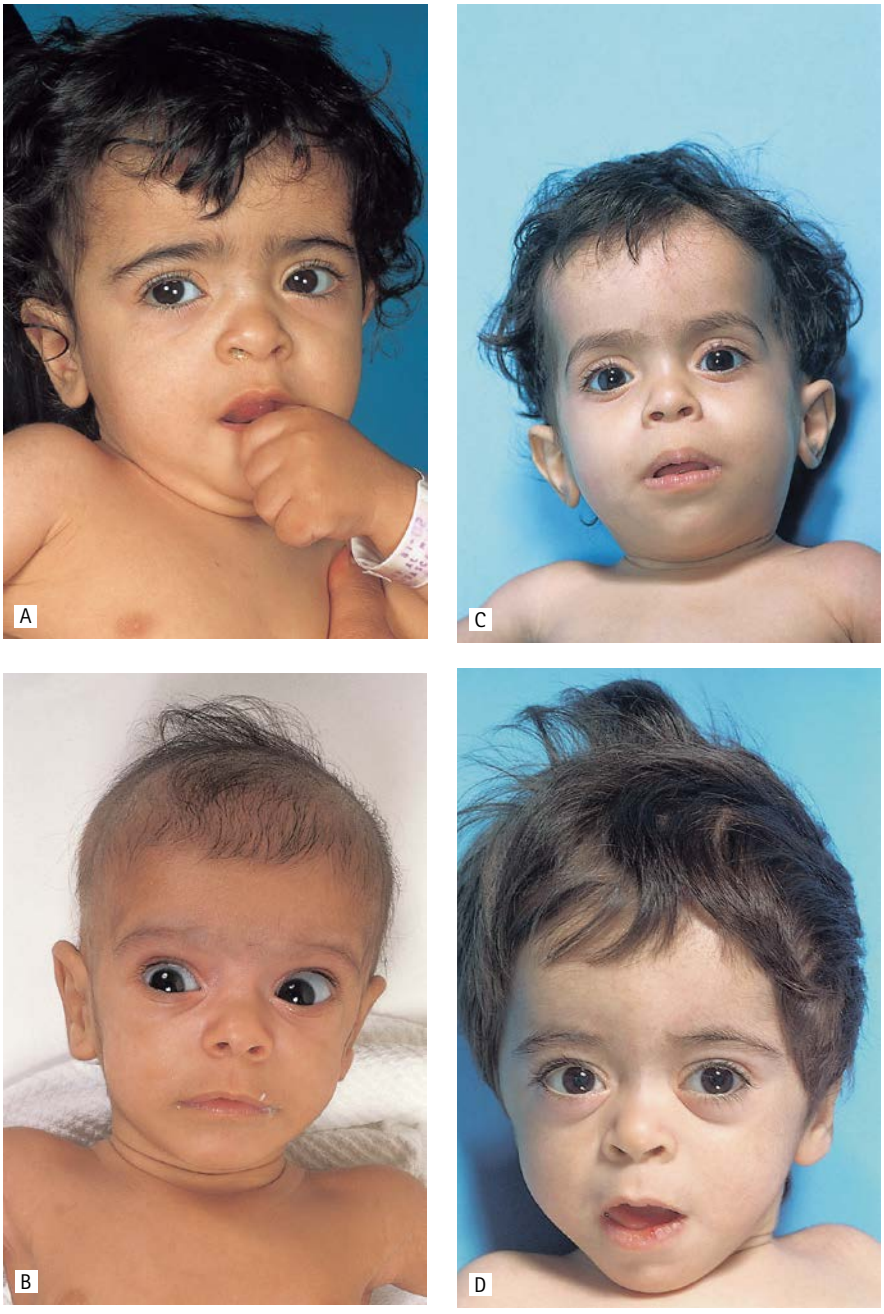

Figure 91.9 Four Saudi infants (A–D) with Niemann-Pick disease illustrating some similarity of facial features. Patients tend to lose adipose tissue over the forehead and about the orbits; the nasal bridge is spared, giving the appearance of a crest of tissue.

in neuronal cells and axons, and cerebral atrophy and neuroaxonal dystrophy are characteristic of type A disease.

GENETICS AND PATHOGENESIS

Niemann-Pick disease is transmitted as an autosomal recessive disease. The disease has been seen widely throughout the population of the world. The frequency of type A is much higher in Ashkenazi Jewish populations in which type B disease is rare.

The molecular defect is in the enzyme sphingomyelinase (see Figure 91.2) [8, 9]. The enzyme was first purified from rat liver [57]. It cleaves the phosphocholine moiety from sphingomyelin. It is a lysosomal enzyme with a pH optimum about 4.5 and a molecular weight of approximately 70 kDa [58, 59]. The cDNA predicts a protein monomer of 64 kDa; if the six potential glycosylations were filled the molecular weight would be 72–74 kDa. There are a number of sphingomyelinase activator proteins (SAPs), but they do not appear to be required for enzymatic activity.

The defect has been demonstrated in tissues such as liver, kidney, and brain [60, 61], cultured fibroblasts [40, 62, 63], and leukocytes [64, 65]. Patients with type A and type B are defective in the same enzyme. Clinical diagnosis is generally based on the assay of leukocytes or fibroblasts. In general, in type A patients, there is less than 5 percent of control activity, and often activity is undetectable.

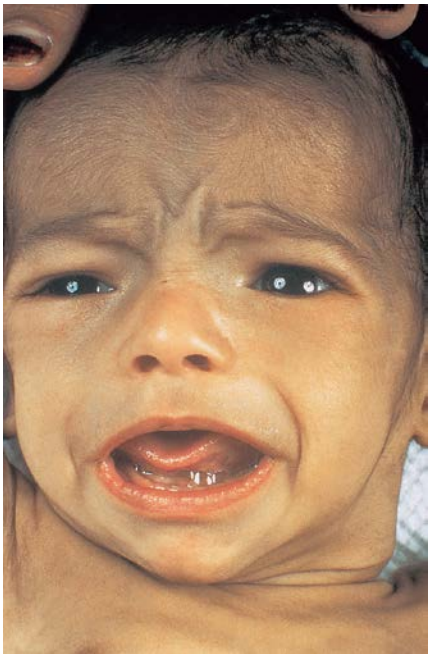

Figure 91.10 Another Saudi infant with Niemann-Pick disease, illustrating a more advanced degree of emaciation of the face. The eyes appear sunken.

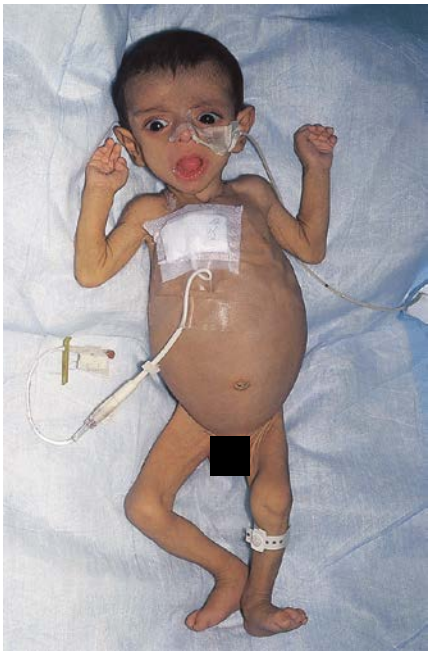

Figure 91.11 This Saudi infant illustrates the extreme emaciation and the large abdomen resulting from organomegaly.

In type B disease, activity is variable and may be up to 10 percent of control [40, 59, 61], but it may also be zero in type B. Residual activity is not a reliable index of clinical severity.

In order to approximate physiologic conditions more closely, a number of investigators have explored intact cell assays in which ^{14}C-labeled or fluorescent natural

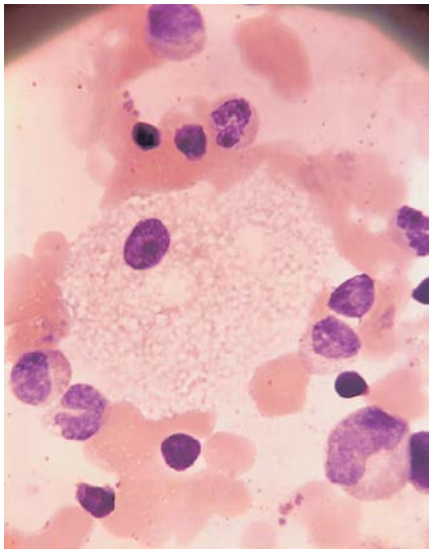

Figure 91.12 The Niemann-Pick foam cells of the bone marrow.

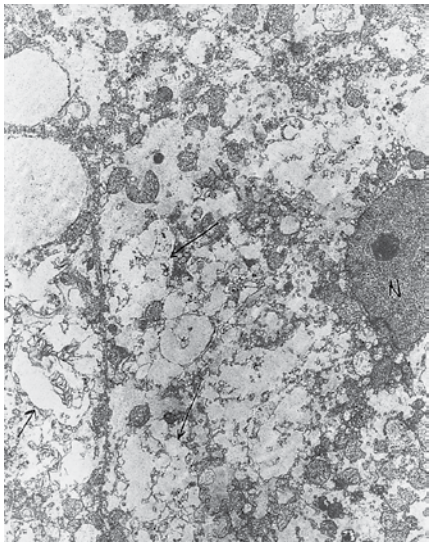

Figure 91.13 Electron microscopy of biopsied liver of an infant with classic infantile Niemann-Pick disease and deficiency of sphingomyelinase. Hepatocytes were large and pale. Electron microscopy, with the nucleus on the right, illustrates many irregular rounded membrane-bound lucent areas. These are considered to have contained lipid, which was extracted (×10,000).

substrate was taken up and transported to lysosomes and then hydrolyzed [40, 63–65]. In these studies, substantial hydrolysis of sphingomyelin was demonstrable in type B cells, while very little occurred in those of type A.

Heterozygotes for types A and B disease generally have enzyme activity that is intermediate between those of homozygotes and normal [13, 66]. In fact, some heterozygotes have had splenomegaly or foam cells in the marrow. On the other hand, heterozygote detection may not be reliably excluded, because of overlap with the normal range.

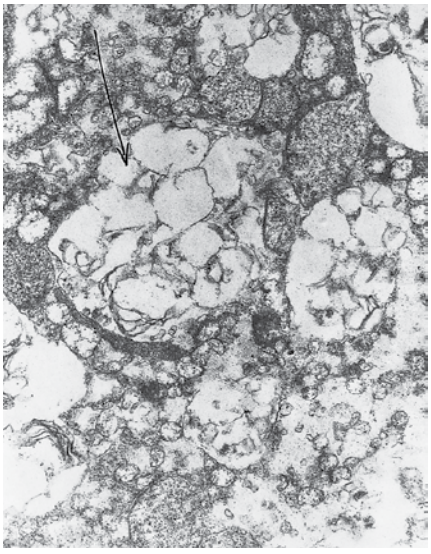

Figure 91.14 In higher magnification (×33,000) the lucent inclusions contained fragments of membranes and granular material.

Prenatal diagnosis has been undertaken by enzyme analysis in cultured amniocytes [40] and chorionic villus material [67], and a number of affected fetuses have been detected [68]. The intact cell assay with labeled sphingomyelin has also been effectively employed with cultured amniocytes [40].

The nature of the enzyme defect leads to the accumulation of sphingomyelin in tissues. This phospholipid is composed of the sphingosine C-18 base with a long-chain fatty acid in amide linkage, forming the ceramide moiety, linked to choline monophosphate (Figure 91.1). Levels in affected patients are enormously increased, especially in tissues rich in reticuloendothelial cells [69, 70]. Type A patients accumulate sphingomyelin in the brain [21, 71], while type B patients generally do not [21]. There is also storage of cholesterol in the liver of patients [69], and this tends to be more in tissues of type A than of type B patients. The other compound that accumulates in the viscera is bis(monacylglycero) phosphate [72].

The molecular genetics of Niemann-Pick disease proceeded rapidly once the gene was sequenced and DNA probes became available. Three common mutations were found in Ashkenazi type A patients [16, 73, 74] that account for 92 percent of the mutant alleles studied [17]. Two are point mutations in exons 6 and 2, R496L (an arginine to leucine change) and L302P (a leucine to proline). The third is a single-base deletion in exon 2 that creates a frameshift (fsP 330) that leads to a stop-codon. The R496L mutation occurred at a CpG dinucleotide, where mutation is common. The two-point mutations have been found in patients homozygous for each and in compounds. The R496L mutation has been found in only one of 20 non-Jewish, type A patients; the other two mutations in none. Interestingly, each of the common type A mutations, R476L, L302P, and FsP330 has been found along with a type B mutation in Ashkenazi patients with a type B phenotype [12, 18], underscoring the artificiality of these old classifications. A small number of mutations has been identified in non-Jewish type A patients, each unique to the family in which it was found [75–78]. Four were single-base substitutions; one a nonsense mutation; one was a single-base deletion which caused a frameshift; and one was a splice site change. Two of the point mutations were in adjacent codons in exon 3.

Among type B patients, a three-base deletion removing an arginine at 608 (DR608) [12, 18] was found in about 12 percent of a large population of type B patients. In homozygous form, patients had mild disease. It also predicts mild disease in compounds with other genes, including the five Ashkenazi Jewish type B patients who carried type A genes in the other allele. This mutation was found in approximately 90 percent of North African Arabs with splenomegaly [79]. In the study of growth restriction in type B children [54], the children homozygous for DR608 were of normal height and weight. The S436R (a serine to arginine) was also associated with mild disease in a 19-year-old Japanese patient [76]. L137P, A196P, and R474W were also associated with mild disease [11]. Among Spanish patients [18], two mutations were common, c.1.823-1825delGCC which accounted for 61.5 percent of type B patients and c.1445C>A (p.A482E).

Expression studies [73, 75] indicated that the DR608 and other mutations found in milder type B phenotype expressed considerable catalytic activity, while mutations that caused premature stop-codons expressed no catalytic activity in COS cells.

Among the Saudi Arabian patients, some 85 percent of alleles carried the two mutations H421Y and K576N [11]; 11 patients were homozygous for the former and two for the latter. These mutations led to early onset and early demise. All had pulmonary disease.

Niemann-Pick type B is relatively common in Turkish patients, in whom three mutations (L137P, fsP189, and L549P) accounted for about 75 percent of the alleles [11]. L137P was consistent with quite mild disease in homozygotes and heterozygotes. The A196P mutation, found to be common in patients of Scottish heritage, appeared to convey mild disease even when in compound with a null mutation. Phenotypic variability was the rule in assessment of 25 percent of Czech and Slovak patients [80]. A majority (69%) had neither of the classic forms but rather a protracted neurovisceral course. The speed or storage intensity were not specific for the classic subtypes which were viewed as an oversimplification.

The phenotype–genotype correlations are useful in counseling the parents of newly diagnosed patients, at least in the populations where mutations are common. They are also useful for prenatal diagnosis and carrier detection in any family in which the precise mutation is known, or in an ethnic group in which a small number of mutations is common.

TREATMENT

A variety of transplantations have been made, including liver and amniotic cells, without evident change. Bone marrow transplantation has been reported as being without effect on the neurologic picture of the type A disease [81], though there may be reduction in the size of the liver and spleen [82]. However, it should be of considerable advantage in type B patients because in type B [83], it has been observed that liver and spleen size decreased regularly following transplantation, and improvement in lung infiltration was documented roentgenographically. Both enzyme replacement and gene therapy are under active exploration, and animal models of sphingomyelinase deficiency are available. A phase 1 study aimed only at the safety of enzyme replacement therapy has begun in the United States [84].

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92

Niemann-Pick type C disease/cholesterol-processing abnormality

MAJOR PHENOTYPIC EXPRESSION

Paralysis of vertical gaze, ataxia, dystonia, hypotonia, prolonged neonatal icterus, hepatosplenomegaly, dementia, foam cells, lysosomal storage of cholesterol, and impaired cellular esterification of cholesterol.

INTRODUCTION

Niemann-Pick type C was first described in the review by Crocker and Farber [1] of their 18 patients with Niemann-Pick disease; the classic features of paralysis of upward gaze, ataxia, dystonia, spasticity, and seizures were clearly described in one of the patients, all of whom had the characteristic foam cells and storage of sphingomyelin. They classified Niemann-Pick into types A and B (Chapter 91), C and D, which is now known to be a variant of C, described in a French-Acadian isolate in Nova Scotia [2]. When the defect in sphingomyelinase was found in types A and B by Brady and his colleagues [3], it became evident that this enzyme was normal in type C [4]. Pentchev and colleagues [5, 6] discovered defective esterification of exogenous cholesterol in the mutant BALB/c mouse. This group then showed that the same faulty regulation of cholesterol processing and storage was present in cultured fibroblasts from patients with Niemann-Pick type C disease [7]. The study of complementation in somatic cell hybrids indicated that type D was allelic with type C on one gene, and clearly separate from types A and B [8].

Complementation studies of type C indicated the presence of two complementation groups [9, 10]. In 95 percent of patients studied, the gene (*NPC1*) was mapped to chromosome 18q11–12 [11, 12]. The smaller group of patients considered to have a defective *NPC2* gene [10] have been reported [13] to have abnormalities in the gene *HE1* on chromosome 14q24.3, which codes for a lysosomal cholesterol-binding protein [14]. A number of mutations in the *NPC1* gene have been found [12].

In Niemann-Pick type C disease, the trafficking of lipid within the cell leads to accumulation of unesterified cholesterol in lysosomes and late endosomes. The NPC1 protein, which is defective in this disease, is a multifunctional protein, normally situated in a unique late endosomal compartment that becomes enriched with low-density lipoprotein (LDL) cholesterol [15, 16]. It is thought that this protein and that of the C2 gene facilitate the egress of cholesterol from late endosomes or lysomes to the Golgi, endoplasmic reticulum and plasma membrane [17–19]. Ultimately, one would hope that this disease would be known by a name that more closely reflects the fundamental defect.

CLINICAL ABNORMALITIES

The classic patient with this disease appears normal at birth, and while there is a fatal form of the disease, and some patients may have neonatal manifestations of hepatosplenomegaly, jaundice, or hepatic dysfunction, isolated splenomegaly may be the only manifestation for as long as seven years before neurologic signs become apparent [20]. The usual onset is with neurologic manifestations between the ages of three and 13 years (Figures 92.1–92.3) [21].

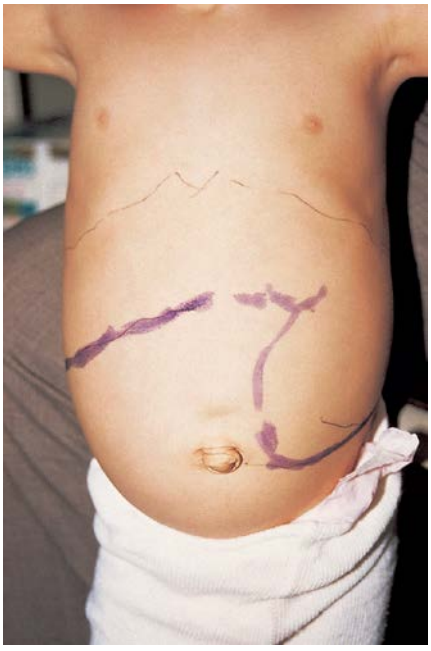

Figure 92.1 A three-year-old with Niemann-Pick disease type C. Illustrated is the hepatomegaly and even more massive splenomegaly. Early development was slow, but she walked by two years; by three years she could no longer walk. She had hypertonia. She died at five years. (Illustration kindly provided by Dr Philip Benson.)

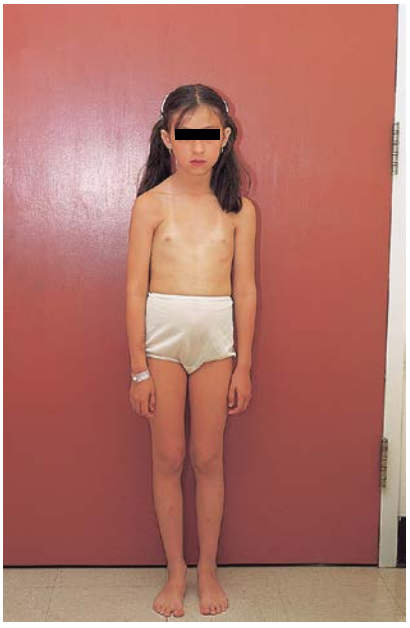

Figure 92.2 GC: A nine-year-old Costa Rican girl with Niemann-Pick disease type C. The first symptom was a tremor at seven years. She then developed ataxia. She had paralysis of upward gaze and weakness of peripheral muscles. Liver and spleen were not enlarged.

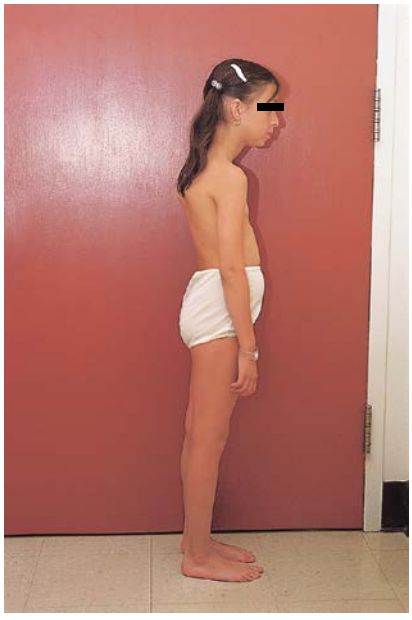

Figure 92.3 GC: Development was at seven years. Storage material was evident in conjunctival cells, and typical Niemann-Pick cells were found in the marrow. Cultured fibroblasts were examined by Dr Roscoe Brady who found defective transport of cholesterol out of the Golgi and lysosomes.

There may be a tremor, clumsiness, or progressive ataxia. School performance may suffer, as ability to concentrate is lost. On examination, the key finding is vertical supranuclear ophthalmoplegia (Figures 92.4–92.6) [22]. Impairment of upward gaze may be the first clinical finding. Downward gaze may also be impaired. Speech is dysarthric. Dystonia develops with posturing on movement and becomes progressively more pronounced and generalized [23]. Seizures may develop and may be difficult to control. Neurologic dysfunction is progressive. Opthalmoplegia may ultimately be complete. Hepatosplenomegaly may be detected in childhood (Figures 92.1–92.3), but with growth this organomegaly may recede. An unusual, not common feature, referred to as gelastic cataplexy, may manifest in nods of the head, or there may be a complete collapse in response to a humerous stimulus [24, 25]. The sudden loss of postural tone may lead to falls and injury. Abnormal behavior may progress to dementia, amentia, or psychosis. The patient becomes wheelchair-bound, and then bedridden. Some have become blind. Early hypotonia may be displaced by spasticity or rigidity. Dysphagia develops, along with drooling and aspiration pneumonia. Death may ensue for these reasons or inanition.

The disease is highly variable in presentation and accordingly difficult to recognize and often diagnosed late. Among the earliest onset, most severe disease, some patients have presented with progressive hepatic dysfunction leading to death well before the onset of neurologic disease [26–29]. Patients have also been reported with fetal ascites [30, 31]; most died in infancy; this disease has been listed as the second most common cause of liver disease in the UK, after α-1-antitrypsin deficiency (Chapter 107) [32]. Of four patients, two died of hepatic failure and another from respiratory failure with foam cells in the interstitial tissue

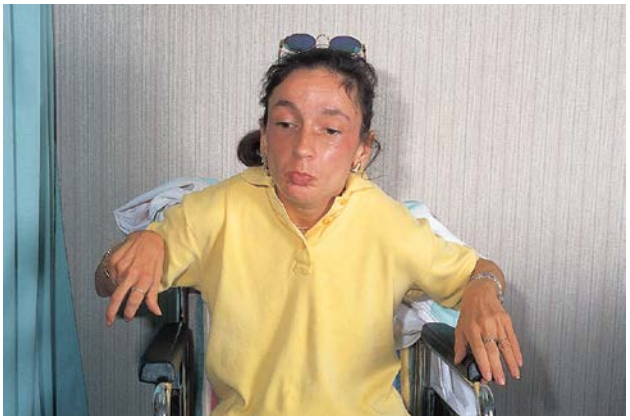

Figure 92.4 GS: A 29-year-old woman with Niemann-Pick type C disease. She was wheelchair-bound, athetoid, dystonic, and dysphagic. She had expressive aphasia. A sibling had died at 32 years of age, and ballooned cells were found in the brain and other organs.

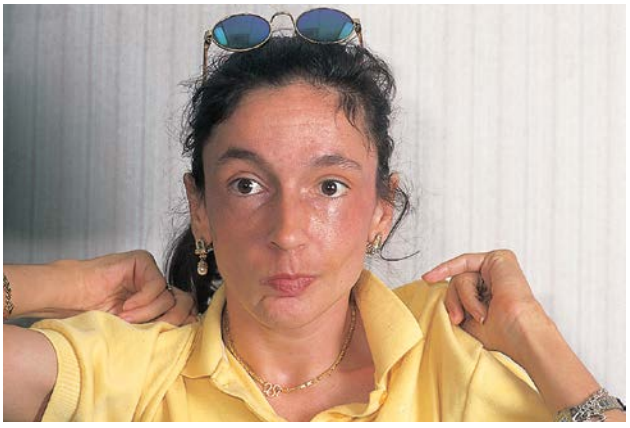

Figure 92.5 GS: Paralysis of upward gaze. She was attempting to look up at the examiner's fingers.

of the lungs [33]. One child presented at four months with respiratory manifestations [20].

Some patients have had delayed development from infancy [25, 34–36], but neurodegeneration began at about three years. Others have had hypotonia.

A variety of hepatic presentations has been observed. The most common is neonatal cholestatic jaundice [21] and this usually disappears spontaneously early, but there may be prolonged, severe jaundice with elevated conjugated bilirubin [37]. Neonatal jaundice has been reported with early-onset rapidly progressive neurologic disease [38]. Others have developed hepatic failure in the absence of neurologic disease.

A number of late-onset or adult presentations have also been reported [22, 39–44]. Some of the patients have had exclusively psychiatric symptoms, psychosis, or progressive cognitive loss and dementia. Most adults have developed paralysis of vertical gaze, but some have not, and some have complained that their eyes became stuck on looking

Figure 92.6 GS: Ophthalmoplegia also involved inability of downward gaze. The hand illustrates the athetoid movement induced by the effort. She could still move her gaze to each side.

up. Others have developed ataxia and pyramidal and extrapyramidal signs, and the neurologic picture of the classic disease [44]. Cerebral atrophy may be evident on neuroimaging. Some have had neonatal hepatitis and then seemed to be well until the development of psychotic symptoms in adolescence or adulthood [43]. A past history of splenomegaly may be another clue to the nature of the disease. Some adults have had a nonneuropathic presentation [43, 44], including a man whose large spleen was ruptured in a traffic accident at 46 years and found to contain foam cells [44]; four years later he was asymptomatic and had a normal neurologic examination.

A fetal form of the disease was reported [45] in five families, three of them consanguineous. Two were diagnosed prenatally by study of amniocytes after developing splenomegaly and ascites. Two had an affected sibling. Prognosis was bad. One died *in utero*; one was terminated during pregnancy; four died within seven months of cholestatic disease, and the seventh had rapid neurologic deterioration at ten months.

The phenotype of patients with NPC2 disease appears to be indistinguishable clinically or biochemically from the more common type 1 disease, but severe pulmonary involvement has been observed [46].

The diagnosis of a neurolipidosis is usually made on the basis of the characteristic foam cells. The usual source is a bone marrow aspirate [22, 47, 48]. Sea-blue histiocytes may be seen [47], as well as the foam cells (Chapter 94, Figure 94.10). The cells stain with periodic acid-Schiff stain, and strongly so with the Schultz stain for cholesterol and the acid phosphatase stain [48–53]. Foam cells may be found on conjunctival biopsy and skin biopsy [54]. In the electron microscope, there are numerous concentric electron-dense inclusions and electron-lucent vacuoles [39, 49, 50, 55].

Pathologic examination indicates the presence of foamy storage cells, particularly in the liver, spleen, tonsils, and lymph nodes [51, 52]. Early infantile-onset disease is associated with an appearance of giant cell hepatitis and cholestasis [26, 29, 38, 39].

Storage in neurons is seen widely in the cerebrum, the cerebellum, and the retina [50, 56–58], and the brain is atrophic. Lamellar inclusions may resemble the membranous cytoplasmic bodies of the gangliosidosis or zebra bodies. Crystalline structures were observed [59] in a 20-week-old fetus, suggesting crystalline cholesterol. Similar structures were found in a murine model of the disease [60].

GENETICS AND PATHOGENESIS

Niemann-Pick type C is an autosomal recessive lysosomal storage disease [61]. Despite considerable clinical variability, one complementation group represents a majority of the patients [9, 10, 61]. The gene (*NPC1*) was mapped to chromosome 18 (q11–12) [11]. In a study of 32 unrelated patients [10], 27 fell into this NPC1 group. Five patients fell into the second NPC2 group. Patients in the first group illustrated the entire spectrum of disease. In general, phenotypes within any family were quite similar, except that fatal neonatal disease was found in families in which others had classic neurologic disease [61].

The disease has been seen widely throughout the world [62]. A genetic isolate of French-Canadians in Nova Scotia was originally called type D [2]. In their county, the incidence was 1 percent, and carrier frequency was 10–26 percent [63]. Another isolate was found in Hispanic-Americans in south Colorado [47]. In France and England, the disease was found to be as frequent as Niemann-Pick types A and B combined [64].

The *NPC1* gene contains 25 exons over 47 kb [65]. It predicts a protein of 1278 amino acids [12]. The protein appears to be a permease which acts as a transmembrane efflux pump [14]. It has extensive homology to other proteins, including the murine ortholog, and to patched, the defect in the basal cell nevus syndrome [66], which is also related to the sonic hedgehog signaling pathway; and to proteins involved in cholesterol homeostasis. Eight mutations were originally found in five patients, two deletions, one insertion, and five missense mutations. In Japan, mutations identified included two splicing abnormalities [67]. In the Nova Scotian French isolate the defect was a missense mutation c.G3097T which led to p.G992W [68]. A considerable number and variety of unique mutations have now been found [69]. The only common Caucasian mutation is p.I1061T, when homozygous leads to a juvenile neurologic phenotype [70]. This mutation has been found in the Hispanic-American isolate in Colorado and New Mexico. In most populations, compound heterozygosity is the rule [71].

The gene for NPC2 was mapped to chromosome 14q24.3 [72]. In studies of the epididymal secretory protein HE1, it was reasoned from the facts that it bound cholesterol and was lysosomal and that it might be relevant to Niemann-Pick C disease. Activity of HE1 was found to be undetectable in fibroblasts from patients with NPC2. Mutations were found in the HE1 gene. Homozygous mutations were found in six patients with NPC2 [73]. Among the mutations, only p.P1205 led to detectable quantities of immunoprotein. IVS1 +2t>c led to a number of transcripts, none of them normally spliced. Genotype phenotype correlations appeared to be good in 22 families with mutations.

Animal models of Niemann-Pick type C disease have been found: a feline and two murine models [5, 6, 74]. The mice were ataxic and had typical foam cells like those of human patients. There was marked cerebellar loss of Purkinje cells.

The fundamental defect in the mice and in humans was in the transportation system for cholesterol in cells [5–7]. Cultured fibroblasts of patients and affected mice were deficient in their ability to make cholesteryl esters from endocytically taken-up exogenous LDL cholesterol [6]. This leads to lysosomal accumulation of cholesterol [7, 75–77]. The enzymes involved in cholesterol esterification, such as acyl-CoA:cholesterol acyl transferase (ACAT), are normal in these cells, and treatment of the cells with 25-hydroxycholesterol reverses the abnormality of cellular regulation of exogenous cholesterol [78].

The abnormality is conveniently demonstrated with filipin, a fluorescent probe that detects unesterified cholesterol [79]. Following LDL uptake by type C cells, the lysosomal vacuoles light up; this is the procedure that has facilitated complementation studies [10]. Endogenously synthesized cholesterol is processed normally because it does not end up in lysosomes [17]. These observations served to focus attention on systems for transport out of lysosomes.

The diagnosis of Niemann-Pick type C disease is currently made in cultured fibroblasts by demonstration of both impaired cholesterol esterification and the positive filipin test for accumulation of free-cholesterol [80–82]. A considerable amount of heterogeneity has been observed in these tests, ranging from mild to severe changes [83]. A majority of patients (86 percent) have cholesterol esterification rates less than 10 percent of normal [81]. Some are very mildly affected and some intermediate, but correlations of this biochemistry with phenotype have not been clear. Assay by filipin staining is more broadly effective, particularly in the diagnosis of variant patients [21, 81]. It is also more specific, because abnormal esterification occurs in other disorders, such as I cell disease (Chapter 83), familial cholesterolemia (Chapter 84), and acid lipase deficiency.

An aid to diagnosis may be obtained by assay of the activity of chitotriosidase [43]. This activity is significantly increased in Gaucher disease [84]. It may be moderately elevated in Niemann-Pick type C disease and some other lysosomal storage diseases, but it may be normal too [43].

Heterozygote detection is unreliable, although some have foamy cells in marrow or skin biopsies [85, 86] and intermediate levels of cholesterol esterification are found in about half of obligate heterozygotes [21, 81, 82, 87]. Prenatal

diagnosis has been carried out by biochemical testing in cultured amniocytes and chorionic villus cells [88, 89]. Thirteen affected fetuses were found in 37 pregnancies at risk [89]. Only the families with the most severe chemical expression appear to be reliable candidates for biochemical prenatal diagnosis. The extensive molecular heterogeneity makes mutational analysis formidable, except in population isolates or in families in which the mutation is known. In these instances, this is the method of choice for heterozygote detection and prenatal diagnosis [69, 71].

The adenosine triphosphatase (ATP)-binding cassette transporter A1 (ABACA1) is also upregulated in response to increased cellular cholesterol, leading to high-density lipoprotein (HDL) particle formation. Mutations in this ABACA1 lead to increased intracellular cholesterol and very low levels of HDL in Tangier disease [90]. Mutations in NPC1 appear to impair also the regulation and activity of ABACA1 [91]. Fibroblasts from patients with NPC disease were shown to have decreased efflux of labeled LDL-cholesterol mediated by apolipoprotein A-I. These fibroblasts also displayed diminished ABCA1 mRNA and protein in both basal and cholesterol stimulated states. Furthermore, 17 of 21 patients studied had low levels of HDL-cholesterol. This observation can provide another diagnostic aid in evaluating children for NPC disease.

Despite the large and increasing body of knowledge about the metabolism and transport of cholesterol and other lipids, the pathogenesis of the neurologic features of Niemann-Pick type C disease remains obscure [92]. Experience with cholesterol-lowering drugs and bone marrow transplantation in man, and even combined liver and bone marrow transplantation in the mouse model, none of which were effective in influencing the neurodegeneration [93, 94], indicated clearly that the central nervous system (CNS) is autonomous from the rest of the body. Liver transplantation in a seven-year-old cirrhotic girl restored hepatic function, but failed to reverse neurologic deterioration [95]. A variety of lipids in addition to cholesterol accumulate in the brains of patients with this disease. These include GM2 and GM3 gangliosides, glucosylceramide, and lactosylceramide. There is neuronaxonal dystrophy and neurofibrillary tangles, like those of Alzheimer disease. In Niemann-Pick type C lipid rafts, which occur in the lipid bilayer of the plasma membranes of glia and neurons, accumulate because of defective egress. Approaches to reduce the accumulation of sphingolipid by inhibiting its synthesis have been underway in murine models, and human trials are planned. N-butyldeoxynojirimycin inhibits the synthesis of GM2 ganglioside [96] and has resulted in reduced ganglioside accumulation in brain, reduced Purkinje cell loss, and modest delay in neurologic disease and death. Similarly, breeding affected mice with mice carrying a mutation in the transferase gene that inhibits synthesis of GM2, GA1, and GA2 indicated that these lipids are not the cause of the neuropathology [97]. Disordered trafficking of liprotein cholesterol leads to disordered oxysterol and sterol biosynthesis. In homozygous NPC1 knockout mice, treatment with allopregnanolone and an oxysterol ligand delayed the onset of neurologic disease [98].

TREATMENT

Specific treatment is not available. The promise of gene therapy was raised by successful prevention of neurodegeneration and extension of lifespan in homozygous npc mice by overexpression of the NPC1 gene targeted to the CNS [99].

Seizures may be controlled with the usual anticonvulsant agents. Protryptilene and clomipramine are useful in cataplexy and sleep problems [100, 101]. Dystonia and tremor may respond to anticholinergic drugs. Supportive care including physical and occupational therapy is important. Support groups are available in the United States and in Europe.

A therapeutic trial of butyldeoxynojirimycin (miglustat), found to prolong survival in mice [96], is underway in man. Preliminary data appear to show some benefit [102].

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93

Krabbe disease/galactosylceramide lipidosis/globoid cell leukodystrophy

MAJOR PHENOTYPIC EXPRESSION

Rapidly progressive central nervous system degenerative disease, characterized by spastic quadriplegia, blindness, deafness, peripheral neuropathy, and pseudobulbar paralysis; diffuse demyelination; massive infiltration with multinucleated globoid cells, thickening of nerves, deficiency of galacto- cerebrosidase; and mutations in the *GALC* gene.

INTRODUCTION

The syndrome was first described by Krabbe in 1916 [1]. He reported five patients, of whom four represented two sets of siblings. All were normal at birth, but had rapidly progressive neurologic deterioration from an early onset at 4–6 months until death by the age of 1.5–2 years. In addition to a detailed description of clinical features of the disease, he clearly documented the pathognomonic neuropathologic features of the disorder, including the accumulation of large multinucleated globoid cells. Chemical analysis documented the accumulation of cerebroside in these cells [2, 3] and the induction of globoid cells uniquely by the intracerebral administration of galactocerebroside [4, 5]. The enzymatic defect (Figure 93.1) was discovered in 1970 by Suzuki and colleagues [6, 7], in galactocerebrosidase (galactosylceramide-β-galactosidase) (EC 3.2.1.46). The cDNA has been cloned [8], and the gene was mapped to chromosome 14q24.3–32.1 [9, 10]. A considerable number and variety of mutations have been identified [11]. A single mutation, a 30 kb deletion (502Tdel) has accounted for a large number of Northern European, US, and Mexican patients [12, 13].

CLINICAL ABNORMALITIES

Patients with the classic infantile form of the disease appear normal at birth, and they develop normally for the first few months. The first symptoms usually appear between three and six months of age [1, 2]. The earliest manifestations are often irritability and bouts of crying or screaming without apparent cause. The neurodegeneration is then rapidly progressive (Figures 93.2–93.6). Universal rigidity

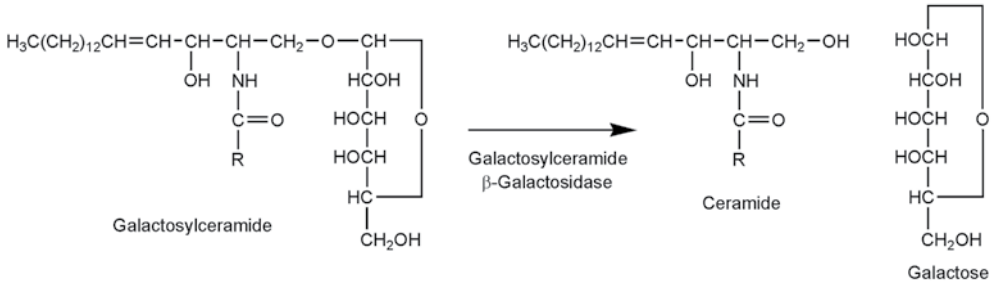

Figure 93.1 The structure of the galactocerebroside, galactosylceramide and the reaction catalyzed by its β-galactosidase. This is the site of defect in the Krabbe disease.

Figure 93.2 SA: A 10-month-old infant with Krabbe disease. This was a modified tonic neck reflex. She was very irritable and had hypertonia. Deep tendon reflexes were exaggerated. She had begun to have trouble handling her secretions.

Figure 93.3 SA: The same infant at five months had begun to manifest developmental delay, but appeared alert and happy. Bifrontal diameter was narrow.

of the muscles is the most typical appearance of the patient with this disease. Fists are clenched and the legs extended. An occasional patient is stiff from birth, and there may be irritability and twitching [14, 15]. Vomiting may be an early symptom [16].

Patients are hypersensitive to sound, light, or touch, and these stimuli set off screaming and rigidity. There may be unexplained fever or convulsive seizures [1, 17]. Some impairment or regression of psychomotor development may be evident early. The level of protein in cerebrospinal fluid (CSF) is elevated at the time of first symptomatology [16], and electrophoresis indicates increase in albumin and decrease in β-globulins. In the

Figure 93.4 SA: At nine months. The expression was blank and the fists clenched.

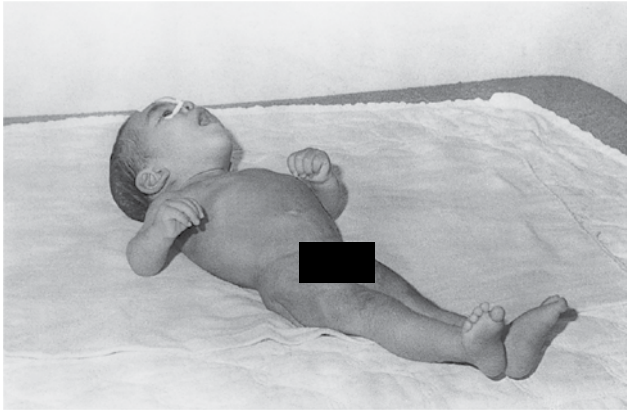

Figure 93.5 MC: A nine-month-old infant with far advanced manifestations of the Krabbe disease. The body was stiff throughout and the hands clenched.

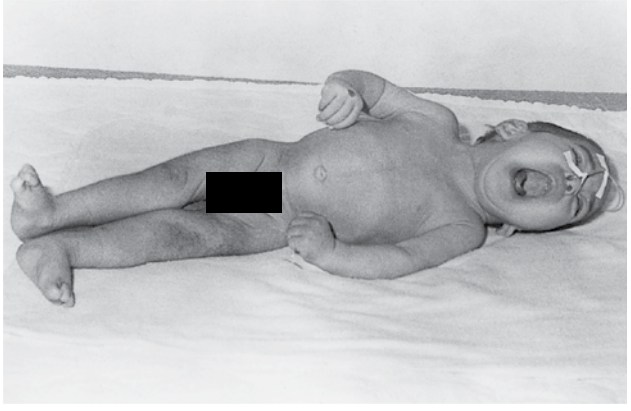

Figure 93.6 This view at nine months illustrates the spasticity, opisthotonus, and clenching of the hands.

second stage of progression [17] the patient is rigid, in opisthotonos, with the head bent well back. The upper extremities are flexed at the elbows, and the hands are clenched. The lower extremities are usually extended at

the hips, knees, and ankles and they are adducted so much that they cross. This may ultimately become the patient's constant position. Deep tendon reflexes are diminished. Motor and mental deterioration is rapid. Mild pallor may be seen in the optic disks, and the pupillary response to light may be sluggish. Convulsive seizures may be tonic, clonic, or myoclonic.

The third stage, by 9–12 months [17], is one of decerebrate blindness, deafness, and flaccidity (Figures 93.5 and 93.6). These patients lose all contact with their surroundings and require tube feeding. Cherry red macular spots have been reported by 13–17 months [18]. Death occurs around two years of age, usually from aspiration pneumonia. Frequent vomiting may lead to malnutrition, as well as aspiration and pneumonia. One patient was admitted to hospital at eight weeks with failure to thrive, feeding problems, and weakness [15]; there were seizures, and deterioration was rapid to death at 15 weeks. Recurrent fever of unknown origin is common. The stiffness of the muscles is always greater in the lower extremities.

These patients often have microcephaly, but macrocephaly has been observed [18–21], as has hydrocephalus [22]. They have no hepatosplenomegaly or bony abnormalities. One patient had ichthyosis [20]. Protruding ears have been described as a feature of the disease [23].

Peripheral neuropathy may not be recognized clinically, but the knee jerks may be observed to disappear [1, 19, 24, 25]. A segmental demyelination of the peripheral nerves is seen [26] and nerve conduction velocity is decreased [21, 23]. In one patient, diagnosed prenatally, neurologic examination was normal in the neonatal period, but deep tendon reflexes were absent by five weeks [27]. By seven weeks, peripheral nerve conduction velocity was abnormal. Psychomotor development was normal for two months; weakness of neck muscles was first found at three weeks. Enlargement of the optic nerves has been reported [28]. Enlargement of the nerves of the cauda equina has been demonstrated by MRI [29]. Elevation of the protein concentration of the CSF may be helpful in suggesting the diagnosis. The electrophoretic pattern of the CSF protein in which albumin and α-globulin are increased, while β-and γ-globulin are decreased, is also seen in metachromatic leukodystrophy.

Nonclassic or late-onset forms of Krabbe galactosylceramide lipidosis have been recognized increasingly since the advent of enzymatic diagnosis [30–33]. Heterogeneity of phenotype has been considerable. Most have presented by ten years of age, but in others neurologic signs developed between ten and 20 years, and one was reported at 39 years of age [31, 34, 35]. In the late infantile group of patients in whom the onset was between six months and three years [36], the manifestations and progression were little different from the classic disease, and death usually ensued within two years of onset. In a second group, in whom the onset was three to eight years [36], the progression was slower, and none had died in the period of follow up, which was as long as seven years. Some were developmentally delayed before the onset of deterioration [32, 37]; some had seizures [38–40]; and two had hemiparesis, progressive in one to tetraplegia [41]. Onset with ataxia has been observed [31, 32]. Adult patients have been described in whom onset was between 10 and 35 years of age. The CSF protein is abnormal in the late infantile patients, but may be normal or only slightly elevated in juvenile or adult patients [34, 35, 42]. Adult-onset patients are being increasingly reported [33, 43] with progressive spastic paraparesis or peripheral neuropathy. Others have had dementia.

In classic Krabbe disease and its variants, neuroimaging usually indicates diffuse cerebral atrophy [44–46]. The scan may be normal early in the disease [47]. Diffuse hypodensity of the white matter has also been described [48]. Plaque-like high intensity T_2 signal has been observed in periventricular and cerebellar white matter in three patients [49].

The electroencephalogram (EEG) is disorganized and slow [14, 19, 20], and there are paroxysmal discharges. There may be asymmetry. The electromyogram (EMG) may be abnormal, and there may be fibrillations [19, 23, 24]. Motor nerve conduction velocity is regularly decreased [19, 23, 25]. In 82 percent of 27 patients, one day to eight years old, there was uniform slowing of sensory and motor nerve condition [50]. The patient may have hyperactive deep tendon reflexes, while electrophysiologic studies indicate a prominent peripheral neuropathy [24]. Among adult patients nerve conduction may be normal [34], or there may be EMG evidence of demyelinating neuropathy [35]. Visual or auditory evoked responses may be abnormal [35]. The former are abnormal early, while the latter abnormalities occur later. In 20 early-onset patients, most had abnormal brain stem auditory evoked potentials (BAEP) and visual evoked potentials (VEP); and 65 percent had abnormal EEGs [51] while less than half of later patients had these abnormalities. Palatal myoclonus has been described in this disease [52].

The neuroanatomic pathology of Krabbe disease is characterized by an extreme hardness or sclerosis of the white matter. Prior to the availability of enzymatic assay, the diagnosis was often established antemortem, by biopsy of brain, which revealed diffuse loss of myelin, astrocytic gliosis and the hallmark finding of a massive infiltration with the multinucleated globoid cells (Figure 93.7) in the white matter [1, 19, 53, 54]. These large irregular cells range from 20 to 50 microns in diameter and contain as many as 20 nuclei. The ultrastructure of the globoid cells reveals abnormal tubular crystalloid inclusions [14, 19, 21, 54]. The same inclusions in globoid cells have been produced in rats by intracerebral injection of galactocerebroside [55]. These observations suggested that the cells accumulate galactosylceramide, and this has been documented by chemical analysis [2]. Peripheral nerves appear grossly thick and chalk-white [19, 26]. Histologically, there is endoneurial fibrosis and complete loss or thinning of myelin sheaths [25].

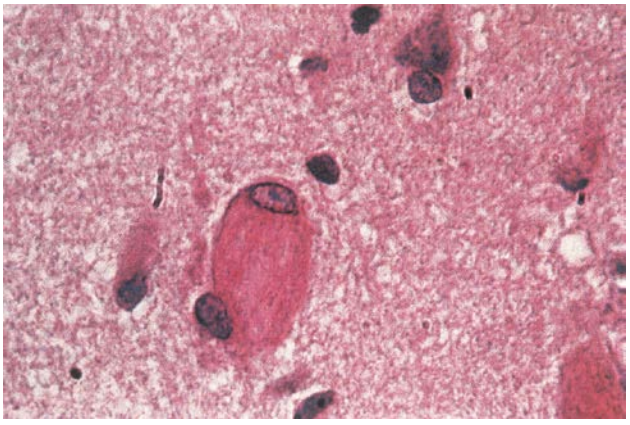

Figure 93.7 The globoid cell that is the hallmark of Krabbe disease. Section was taken from the brain of MC.

GENETICS AND PATHOGENESIS

The disease is transmitted as an autosomal recessive trait [56]. Multiple siblings have been reported with normal parents. In Krabbe's original report there were two sets of siblings [1]. Parental consanguinity has also been observed. There is no ethnic preponderance, and the disorder has been seen throughout the world. In a report from Israel [23] all the patients were Arab. The disease appears to be common in Scandinavia; the incidence in Sweden was calculated to be 1.9 per 100,000 births [56], and in Japan, the estimate was one in 100,000–200,000. The parents of patients have been found to have enzyme activity that is distinctly lower than normal and higher than in patients [57]. However, carrier detection is not always reliable, because values in some carriers may overlap the normal range. Prenatal diagnosis of an affected fetus was first reported in 1971 [58]. It is now possible by enzymatic assay of chorionic villus material, as well as amniocytes. It is recommended that enzyme assay be carried out on parents before prenatal diagnosis is undertaken to avoid a false positive in the case of a very low value in a heterozygote. Once the mutation is known, molecular diagnosis may be carried out for heterozygosity and prenatal diagnosis.

The structure of galactosylceramide is shown in Figure 93.1. Cerebrosides are monohexosyl ceramides in which the sugar is glycosidically linked to the C-1 of ceramide. Galactosylceramide is the characteristic cerebroside of myelin and of the central nervous system. The compound is normally degraded to ceramide and galactose by the lysosomal enzyme galactosylceramide β-galactosidase [6]. In patients, the level of activity has been documented to be 5–10 percent normal in brain, liver, spleen, and kidney [6, 7]. The assay is conveniently and reliably performed on leukocytes or cultured fibroblasts [59]. Enzymatic diagnosis with the natural substrate is demanding and should be carried out in an experienced laboratory [57, 60].

A mutant allele has been reported [61] in which the galactosidase activity overlaps that of patients with Krabbe disease. The proband of the first family was a healthy public health nurse who had volunteered as a control in a study of Krabbe disease. Her leukocyte enzyme activity was consistently lower than 10 percent of control. The presence of this new allelic gene could lead to a misdiagnosis of Krabbe disease, especially *in utero*. The situation could be like that of the Duarte variant for galactose-1-phosphateuridyltransferase, in which compound variants have been observed who were heterozygous for both the gene for galactosemia and that for the Duarte variant. These findings reinforce the recommendation to establish the enzymatic profile in parents before undertaking a prenatal diagnosis. Enzymatic assay does not distinguish infantile from late-onset forms of the disease. Methodology has been developed for dried blood spots in which the product is assayed by tandem mass spectrometry, which permits newborn screening [62–64]. In New York, five infants were diagnosed in almost 2×10^6 tested [64]. These five were early onset.

The twitcher mouse has an autosomally recessively determined deficiency of galactosylceramide β-galactosidase and is an interesting model for Krabbe disease [65]. Other models have been found in West Highland and Cairn terriers, sheep, and monkeys. In the mouse the gene has been mapped to chromosome 12 [66].

The cDNA for the GALC gene contains 3795 base pairs and codes for 669 amino acids [8]. There are 59 and 39 untranslated regions. Expression has been documented by transfer to COS-1 cells.

A rapid test of genomic DNA for the common 502T/del mutation has been developed [13]. In Holland, this accounts for 50 percent of mutant alleles [67]. Two other mutations, p.C1538T and p.A1652C, are relatively common in patients of European ancestry [11, 67]. In Israel, homozygosity for c.T1748G (p.I5835) is found in the Druze population, and c.C1582T (p.D528N) in Arab Muslim patients [11]. Among late-onset patients, c.G809A is relatively common. A polymorphism, c.T1637C, which reduces activity slightly, is found on one allele and a disease-causing mutation, such as c.502T/del or c.G809A on the other in some late onset patients [68]. In 17 Japanese patients, six novel mutations were reported [69]. Two were nonsense (p.W115X and p.R204X). They observed that 12del3ins and p.I66M and I289V, which have been found only in Japanese, accounted for 37 mutant alleles, and with p.G270D and p.T652P accounted for 57 percent of mutations in Japanese patients.

The pathogenesis of disease in galactosylceramide lipidosis is not clear. It is an unusual lipid storage disease, in that the stored substrate accumulates only in globoid cells. Storage cannot be demonstrated in lysosomes. The disease in the mouse differs in that inclusions are seen, and the cerebroside accumulates in both kidney and lymphocytes.

In both mouse and man, levels of psychosine were increased in brain and peripheral nerves [70, 71]. This compound, galactosylsphingosine, which differs from the cerebroside in the absence of the fatty acid, is not present in large amounts, but it is essentially absent from normal brain. The terminal galactose is cleaved from this

compound, too, by the enzyme that is defective in Krabbe disease. Psychosine is a natural detergent and highly toxic [72]. Oligodendroglia appear to be selectively destroyed by psychosine formed within them.

TREATMENT

Effective specific treatment has not yet been devised. Bone marrow transplantation has been performed in a few late-onset patients without clear evidence of efficacy [29, 73, 74], though stabilization of some late-onset patients appears to have been accomplished by hematopoietic stem cell transplantation (HSCT) [75]. Among 11 infantile-onset type patients given stem cell transplantation before the onset of symptoms, they were reported [76] to have normal levels of galactocerebroside in blood, progressive myelination and normal cognitive function in most, but some had delayed development. In two patients who received HSCT [64], moderate to severe developmental delays were reported. The cloning of the gene and the availability of animal models provide avenues for the study of gene therapy [77]. A worldwide Krabbe registry has been established [78].

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94

Lysosomal acid lipase deficiency: Wolman disease/ cholesteryl ester storage disease

MAJOR PHENOTYPIC EXPRESSION

Vomiting, diarrhea, failure to thrive, abdominal distension, hepatosplenomegaly, adrenal calcification, vacuolated peripheral lymphocytes and foam cells in the marrow, dyslipidemia, elevated aminotransferase in serum, storage of cholesterylesters and triglycerides in lysosomes, and deficiency of lysosomal acid lipase. The cholesteryl ester storage disease phenotype is of a later onset progressive hepatic fibrosis that may be progressive to cirrhosis; mutations in the *LIPA* gene.

INTRODUCTION

Wolman and colleagues [1] reported first one, then two more siblings in the same family, in whom the accumulation of cholesterol and triglycerides was associated with abdominal distension, hepatosplenomegaly, and calcification of the adrenals. Death occurred within the first three months of life. The molecular defect in this disease is the lysosomal acid lipase (EC 3.1.1.13) [2]. This lipase, first demonstrated to be defective in the liver and spleen, is a 46 kDa glycoprotein active on both triglycerides and cholesteryl esters (Figure 94.1). The enzyme is also defective in cholesteryl ester storage disease. The two diseases are allelic, caused by mutations at the *LIPA* locus on chromosome 10q23.2-q23.3 [3]. In general, the mutations in patients with Wolman disease are major alterations that lead to absence of enzyme activity [4–6]. Most patients with cholesteryl ester storage disease have at least one copy of a single mutant allele, a G934A mutation at the exon 8 splice junction, which leads to exon skipping and the loss of codons 254–277 [7, 8].

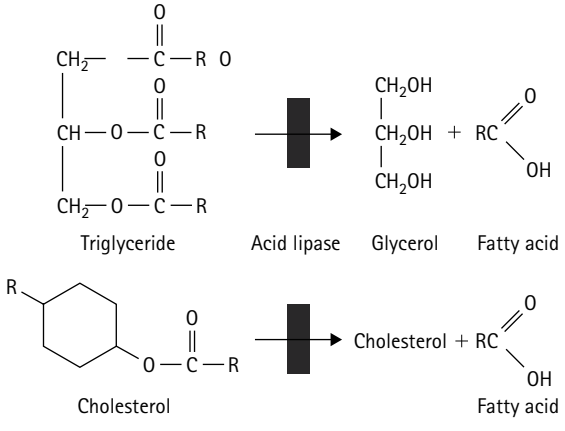

Figure 94.1 Schematic view of acid lipase, the site of the defect in Wolman and cholesteryl ester storage diseases. The enzyme catalyzes the release of free fatty acids from triglycerides and from cholesteryl esters.

CLINICAL ABNORMALITIES

Symptoms of Wolman disease begin in the early weeks of life, and most patients have died by six months of age; survival as long as 14 months has been observed [2]. Infants appear normal for 2–7 weeks; then they develop diarrhea and vomiting [1, 9–11]. This presentation is sufficiently nonspecific that patients are usually thought at first to have gastroenteritis. Stools remain watery and green, and soon

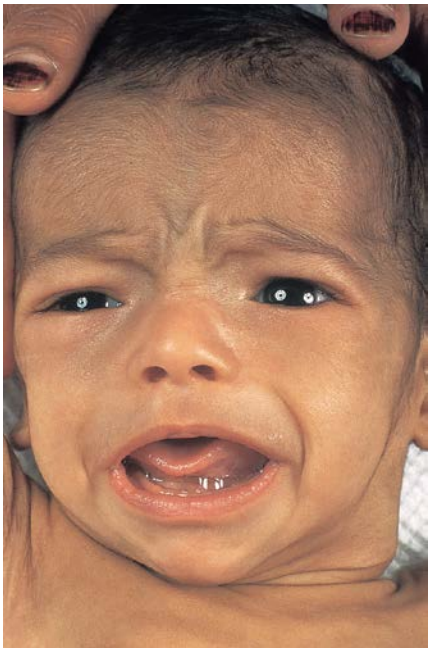

Figure 94.2 SIIS: A three-month-old boy with Wolman disease. The emaciation is evident in the face.

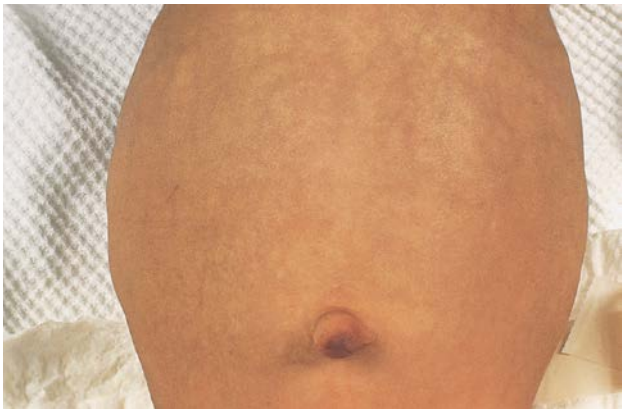

Figure 94.3 The distended abdomen and prominent venous pattern of the same patient. The liver was enlarged. Enzyme assay of cultured fibroblasts revealed less than 5 percent of control activity.

failure to thrive is evident (Figure 94.2). In a few infants, loose watery stools occur in the first weeks of life [12, 13]. As symptoms persist, an intestinal or malabsorption etiology is generally sought. The abdomen regularly becomes impressively distended (Figures 94.3 and 94.4). This may lead to laparotomy in a search for intestinal obstruction [14], or for other reasons [9] (Figure 94.5). The diagnosis of a lipid storage disease will usually be evident on laparotomy because of the appearance of the liver and spleen. Biopsy will confirm the presence of lipid storage. Not all patients come to laparotomy; thus, other clues to the diagnosis must be sought. Affected patients appear wasted and severely ill (Figures 94.2 and 94.4) [9, 10, 14]. Some patients have

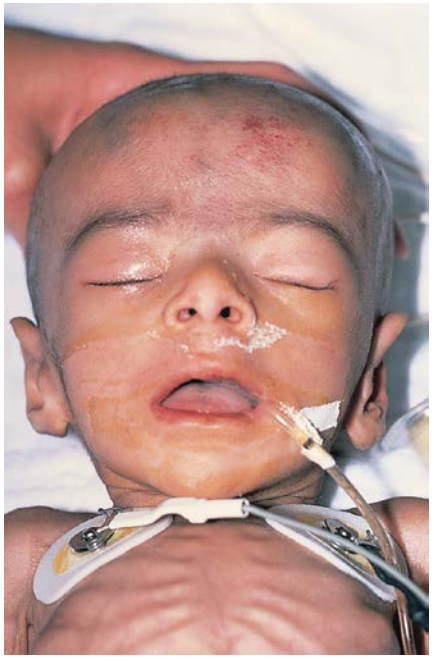

Figure 94.4 IMIS: A two-month-old boy, a previous sibling of the boy in Figures 94.1 and 94.2, also with Wolman disease. He was seriously ill, endotracheally intubated, and died 12 days later. He developed watery, green diarrhea and vomiting at three weeks of age and was admitted to hospital for abdominal distress at 23 days.

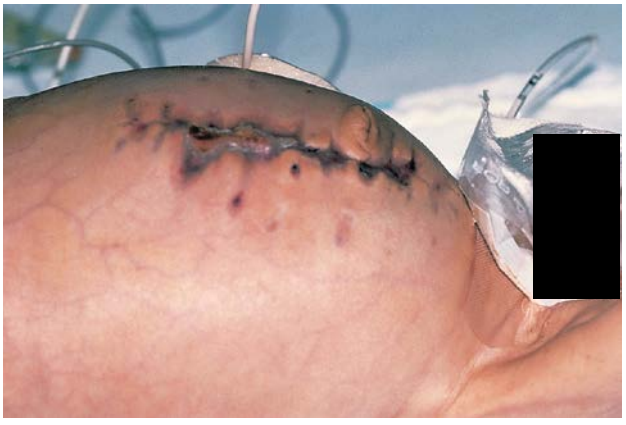

Figure 94.5 Abdominal distension remained massive in this patient following surgery for what had been thought to be intestinal obstruction, and there was dehiscence of the wound. Grossly enlarged and yellow lymph nodes were noted at surgery. Histologic examination revealed lipid storage.

jaundice [10, 11, 15] and some, a low-grade fever [1, 10]. There is impressive, massive enlargement of the liver and spleen [1, 12–14]. Hepatosplenomegaly may be evident as early as the fourth day of life [12] and may be massive.

Calcification of the adrenals is a hallmark feature of this disease [16]. In an infant with the usual clinical manifestations, it should lead to the diagnosis. Calcification may be seen on plain roentgenogram of the abdomen, as fine-stippled or discrete, punctate calcification

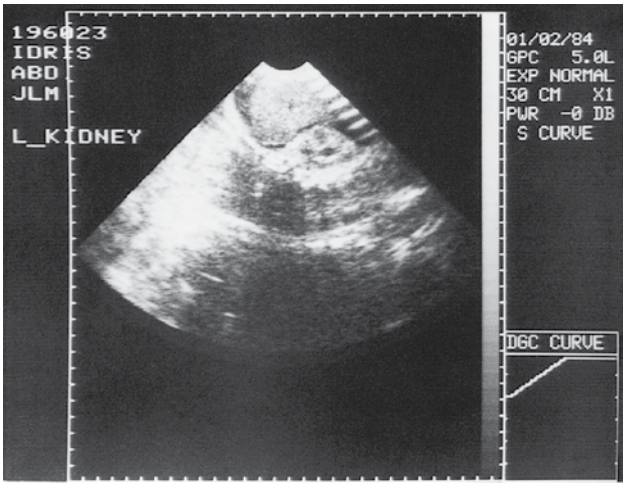

Figure 94.6 Ultrasound of the left kidney shows a dark acoustic shadow resulting from the calcification in the adrenal.

[9, 10]. However, it may be readily missed on routine roentgenograms, especially in the presence of ascites. It is no accident that the most frequently reproduced illustration is of a roentgenogram of adrenals following their removal at autopsy [10]. The calcifications are diffuse, and follow the outline of the glands. This appearance distinguishes these adrenal calcifications from those of adrenal hemorrhage or a neuroblastoma. The earliest appearance may be of enlarged adrenals, which may displace the kidney downward or flatten the superior pole, without deforming the caliceal system or interfering with renal function. Over the next few months of life, the adrenals shrink and become increasingly calcified. The calcifications may be found on ultrasonographic examination (Figure 94.6), in which a dark acoustic shadow is evident. The best way to visualize calcified adrenals is with a computed tomography (CT) scan (Figures 94.7 and 94.8); various cuts permit an estimation

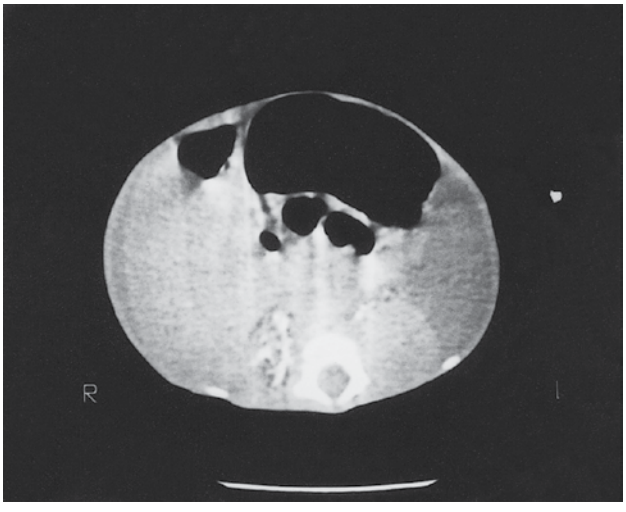

Figure 94.7 CT scan of the abdomen reveals the calcifications in the left adrenal and enlargement of the right adrenal.

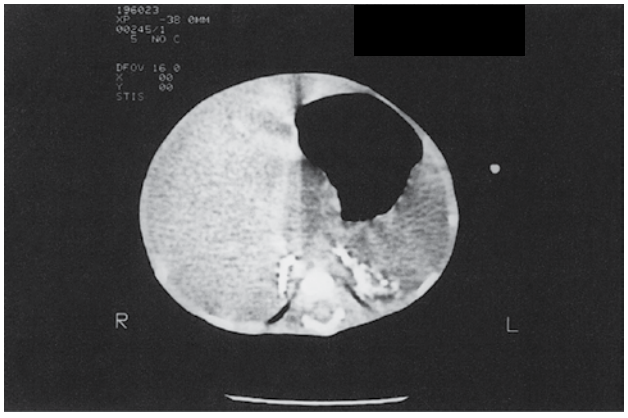

Figure 94.8 In this view, the CT scan clearly shows both adrenals to be densely calcified.

of the size of the adrenals, and the dense calcification is readily evident.

Other roentgenographic features of the disease include the hepatosplenomegaly and/or ascites. The bones are usually hypodense, and there may be a wide marrow cavity and thin cortex, or poor modeling [10].

Anemia is a prominent early feature of the disease [1, 9, 10], usually evident by six weeks. It worsens progressively and may require transfusion. Acanthocytosis has been reported [17]. Thrombocytopenia is not a feature of the disease. Vacuolated lymphocytes or granulocytes (Figure 94.9) may be found in the peripheral blood. The vacuoles are both intracytoplasmic and intranuclear. In many patients, the initial clinical impression is first confirmed by the aspiration of lipid-laden histiocytes from the bone marrow (Figure 94.10). These foam cells are quite similar to those found in Niemann-Pick disease [18], and a number of patients reported as Niemann-Pick disease with adrenal calcifications were probably early examples of Wolman disease. Rarely, phagocytosis of erythrocytes by these cells may be seen [9]. These large, pale, foamy cells may be present in the marrow as early as 40 days. Later, they are present in large numbers and may even be seen in

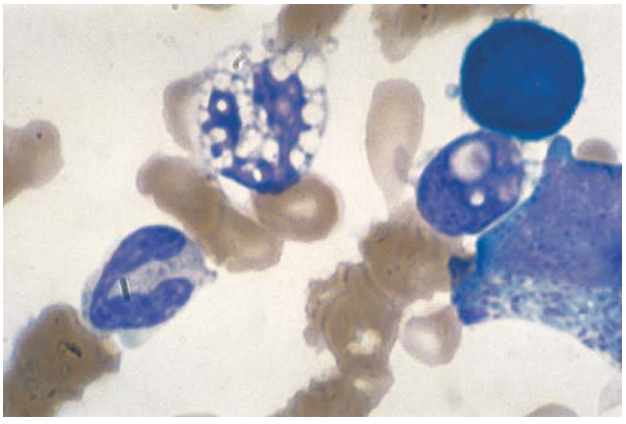

Figure 94.9 Vacuolation in a peripheral blood granulocyte.

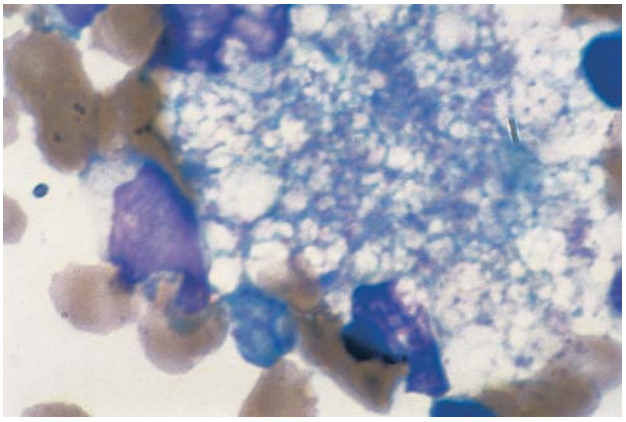

Figure 94.10 Large foamy histiocyte from an aspirate of bone marrow.

peripheral blood [1]. Electron microscopic examination may reveal vacuolation and granular inclusions in circulating granulocytes; vacuoles may be seen on light microscopy.

Psychomotor development appears delayed or to deteriorate, but these patients are so ill that it is difficult to assess whether or not the nervous system is abnormal. Neurologic examination may be normal. Patients are often described as bright and alert but weak [9]. Deep tendon reflexes may be hypoactive or brisk [10], and the plantar response may be extensor, but this may be normal at this age. In one patient [11] deep tendon reflexes were exaggerated, and there was ankle clonus, as well as opisthotonos. The electroencephalogram is usually normal [10, 11, 18].

Plasma cholesterol and lipids may be low or normal [9, 17], or the serum may be hyperlipemic in the fasting state [9], and levels of lipids may be elevated. The lipid is largely triglyceride. The erythrocyte sedimentation rate may be elevated. Liver function tests are usually abnormal [2]. Hypoglycemia may occur as hepatic function deteriorates. Malabsorption can be shown using ^{131}I-labeled triolein [18] or unlabeled fat [12] to demonstrate impaired absorption of fat. Administration of adrenocorticotropic hormone (ACTH) may reveal diminished responsiveness of the adrenals [12].

Pathologic examination [1, 10, 14, 19] of material obtained at biopsy or autopsy showed the liver to be yellow or yellow-tan and greasy on its cut surface. Hepatic architecture is distorted, so that only the portal spaces may be recognizable. Foamy macrophages or Kupffer cells are scattered amid large vacuolated hepatocytes. By the time of death, periportal fibrosis is the rule, and there may be frank portal cirrhosis [10, 12, 20]. Electron microscopic examination discloses well-defined fat droplets bound by a trilaminated membrane and, with the exception of hepatocytes, slender crystals [20]. Most fat droplets are within lysosomes [17]. The endoplasmic reticulum may be distended [21]. The adrenals are large and pale or bright yellow. Calcifications may be felt as gritty on cutting. On section, it is the outer cortex that is yellow; the central zone is gray. The histologic architecture is preserved, but the cells are large, vacuolated, and swollen [9, 10, 14]. Foam cells contain sudanophilic material; some contain birefringent crystals, and occasionally the Maltese crosses typical of cholesterol [12, 20]. Some foam cells become necrotic and it is in these areas that calcification is prominent. It may be condensed in dense crystalline lumps [9]. There may be extensive fibrosis.

The small bowel may be yellow, thickened, and dilated [14, 20], and changes are most marked in the proximal small bowel. Pneumatosis has been described in the colon [14]. Infiltration of the small intestine by foamy histiocytes is extensive, and the mucosal cells are also foamy. These changes appear to account for the malabsorption [14, 21]. In addition, there is infiltration of the ganglion cells of the intestine [14], which may be related to the distension that is so characteristic of these patients. There may also be ileus resulting from potassium losses caused by the chronic diarrhea. The spleen is grossly enlarged, and spleen and lymph nodes largely comprise large, foamy, vacuolated cells. Clear-cut evidence of storage of lipid in neurons of the brain has been reported [14, 22–24]. Swollen glial cells and histiocytes have also been observed. There may be a decrease in the numbers of neurons and impaired myelination [10]. Gliosis of the white matter has been reported [23, 24], but this apparent leukodystrophy may be artifactual [25]. Electron microscopic examination has documented extensive accumulation of lipid throughout the central and peripheral nervous system. In the brain, oligodendrocytes were the major sites of storage.

Cholesteryl ester storage disease

Deficiency of the same lysosomal acid lipase that is defective in Wolman disease is found in cholesteryl ester storage disease [26]. Patients with this disorder have a much more indolent disorder which may present with otherwise asymptomatic hepatomegaly or hepatosplenomegaly in childhood or adulthood [27–33]. Massive splenomegaly and a splenic abscess were reported in one patient [32]. Recurrent abdominal pain has occurred in some patients, and some have had recurrent epistaxis or intestinal bleeding. There may be evidence of cirrhosis on biopsy. Esophageal varices have occasionally been observed [31, 34, 35]. Acute or chronic hepatic failure has been reported in a few patients [35, 36]. Some are icteric. Clotting factors, including prothrombin and factor V, may be reduced. Some patients have hyperlipemia and elevation of the plasma concentration of cholesterol. Pulmonary hypertension has been reported as a complication, leading to death at 18 years [36]. Hyperlipoproteinemia type IIb is commonly encountered, and some patients have xanthelasma. There may be impressive premature atherosclerosis.

In general, patients diagnosed early in childhood have more rapid progression than those diagnosed later. In a summary of 135 patients [37] with cholesteryl ester storage disease 99.3 percent presented with hepatomegaly and

74 percent also had splenomegaly. Elevated AST and/or ALT activities were reported in all. Total cholesterol was elevated in most; while HDL cholesterol was decreased. In a more recent report of 49 patients [38] transaminases were elevated in most; LDL and total cholesterol were elevated in over 60 percent, despite treatment with lipid-lowering therapy.

Common extrahepatic manifestations were diarrhea, abdominal pain, emesis, anemia, muscle malabsorption, cholestasis, steatorrhea, gall bladder dysfunction, and poor growth. Cardiovascular disease was common. Hepatocellular carcinoma was found in two patients. Adrenal calcifications, uniform in Wolman disease were found in about 50 percent of patients.

The reduction in activity of the acid lipase is 50- to 100-fold [38] – severely depressed but much less so than in Wolman disease. On the other hand, in most assays, the difference in activity seen in cholesteryl ester disease is not appreciably different from that of Wolman disease, and certainly not enough to account for the differences in phenotype [26, 39]. However, this is also the case in most attempts to study genetic heterogeneity by enzyme assay in lysates of cells or tissues. Normal amounts of cross-reacting material (CRM) have been found in fibroblasts.

Histologically, the macrophages of the liver are full of cholesteryl esters. A patient was reported to have sea-blue histiocytes in the marrow [40]. Microvesicular steatosis was progressive to cirrhosis [37]. Birefringent cholesteryl ester crystals or their remnant clefts were pathognomonics [37]. Extrahepatic findings included portal hypertension, esophageal varices and atherosclerosis. Hepatic failure led to transplantation in 17 [37, 38].

Monitoring of disease progression is recommended with annual liver function tests, chitotriosidase, and a lipid panel, along with periodic MRI of the abdomen and annual EKG and echocardiography.

GENETICS AND PATHOGENESIS

Incidence of Wolman disease is estimated at 1 in 500,000 [37]. That of cholesteryl ester storage disease has been estimated in 1 in 40,000.

The measurement of lysosomal acid lipase in blood is complicated by the fact that there are other lipases in whole blood. A method has been developed that takes advantage of specific 3, 4-disubstituted thiadiazole carbamate inhibitors, the most effective of which was named Lalistat 2 [41]. The compound has no effect on pancreatic lipase. The method clearly distinguishes normal individuals, carriers and especially patients. Chemical analysis of tissues in both Wolman and cholesteryl ester storage diseases reveals increased quantities of cholesteryl esters and triglycerides [9, 10, 42]. This may be readily demonstrated by thin layer chromatography. A high-performance liquid chromatography (HPLC) method for the quantification of lipids is useful in the differentiation of Wolman disease, Niemann-Pick disease, and Gaucher disease [43]. It may be used with fibroblasts, lymphocytes, or leukocytes, as well as tissue samples. Lipid analysis has most commonly been reported of the liver and spleen, where the triglyceride content may be as much as 10 and 350 times the normal value, and the total cholesterol content is always increased [42]. An eight-fold elevation has been reported in adrenal [12]. Storage of cholesteryl ester has also been documented in fibroblasts [44]. Unusual oxygenated steryl esters such as those of 7-α-hydroxycholesterol have been found in tissues [42].

The defective activity in the acid lipase is consistent with the accumulation of these lipids in tissues. The enzymatic defect is demonstrable in a wide variety of tissues [2, 45], including leukocytes [46, 47] and cultured fibroblasts [26, 38, 48]. Lysosomal acid lipase may be separated electrophoretically into three isozymes: A, B, and C. It is the A isozyme that is defective in Wolman and cholesteryl ester storage diseases [26, 49]. Immunochemical studies using antibodies against normal acid lipase revealed CRM in fibroblasts of patients with both diseases [38]. The amounts of CRM were at the level found in normal cells, while enzyme activity in Wolman disease was reduced 200-fold.

Wolman disease and cholesteryl ester storage disease are caused by allelic recessive genes at the same locus on chromosome 10 [50], causing deficiency of lysosomal acid lipase [34]. Multiple affected siblings of normal parents have been reported in a number of families [11, 51], as has consanguinity [1, 11, 52–54]. Heterozygosity can be detected by assay of acid lipase in leukocytes or cultured fibroblasts [20, 36, 46, 47, 55–57]. Levels are about 50 percent of normal. Prenatal diagnosis has been accomplished in Wolman disease by demonstration of the deficiency of acid lipase in cultured amniocytes [58]. In a family in which the mutation is known, DNA diagnosis may be employed for heterozygote detection and prenatal diagnosis.

The gene for lysosomal acid lipase has been cloned [60] and localized to chromosome 10q23.31 [3]. The gene has been sequenced and contains 10 exons. A number of mutations have been identified [3, 7, 59–62]. The common G934A mutation in cholesteryl ester storage disease leads to a truncated protein missing 24 amino acids [7, 8]. Nevertheless, patients have had a variety of levels of enzyme activity. The cholesteryl ester disease phenotype has also been seen in patients with the common mutation in compound with mutations otherwise found in Wolman disease, such as L179P [4]. The G934A mutation has not, however, been found in patients with the Wolman phenotype.

In the first patient with Wolman disease in whom mutations were identified, L179P was in compound with a frameshift mutation at nucleotide 634 (insT) causing a premature stop (Fs178) [4]. A majority of patients with Wolman disease have been homozygotes, and many had truncating mutations [8, 63, 64]. The common exon 8 splice site mutation at-1 was found to yield 3 percent of correctly spliced mRNA and a full-length enzyme [8]. On the other

hand, sibs with Wolman disease homozygous for a splice site mutation at the same donor site had no correctly spliced mRNA and no enzyme activity.

Over 40 loss of function mutations have been identified [37, 65]. The most common, a splice junction mutation (E8SJM$^{1G>A}$) (c.894G>A) has been found only in cholesteryl ester storage disease. Frequency of heterozygotes in West Germany was one in approximately 200 [37]. Two exon 8 splice junction mutations E8SJM+1G>A and E8SJM+3C>T have been found only in Wolman disease. Of 19 mutations in Wolman disease, 37 percent were small deletions/insertions, and 26 percent nonsense mutations. Of 32 cholesterylesters mutations most (50%) were missense mutations. *In vitro* expression studies have shown that missense mutations causing Wolman disease coded for little or no enzyme activity.

TREATMENT

Patients die by three to six months of age. Cholester ester storage disease is treated with diets low in fat and lipid lowering drugs such as statins and cholestyramine. Despite changes in circulating cholesterol and enzymes, hepatic fibrosis tends to be progressive to hepatic failure and transplantation of liver. Many patients treated with HMGCoA reductase inhibitors went on to transplantation or death [37].

The use of HMG CoA reductase inhibitors to reduce cholesterol biosynthesis and apolipoprotein B generation appears prudent in cholesteryl ester storage disease [8, 65]. Despite bone marrow transplantation and engraftment, one patient died of pulmonary dysfunction, and three others were failures despite successful engrafting in two [67]. However, success has been reported [68], but long-term survival has not been observed [37].

Recombinant human enzyme has been explored [66] as enzyme replacement therapy. A plant preparation has been reported [70] to be effective in a null mouse model. The enzyme known as sebelipase α has been given to nine patients intravenously (IV) over 12 weeks. There were rapid decreases in transaminase levels and decreases in total cholesterol and LDL cholesterol [69]. Initial increases in lipid profiles were consistent with hypothesized mobilization from tissue stores. Twelve patients have received transplanted livers [65]. End-stage renal failure and hemodialysis has not been prevented by transplantation of the liver; nor has vascular deposition of lipid.

Acid lipase replacement therapy has been found safe and effective in 66 patients in a phase 3 randomized double-blind placebo-controlled trial [71]. The primary end point was reversion to normal of ALT. The lipase was administered IV at 1 mg/kg every other week for 20 weeks, followed by 16 weeks of open-label drug. Mean change in ALT from baseline was -58 u/L. LDL and HDL cholesterol improved significantly.

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Fucosidosis

MAJOR PHENOTYPIC EXPRESSION

Progressive mucopolysaccharidosis-like disease with developmental impairment, shortness of stature, coarse features, hepatosplenomegaly and dysostosis multiplex, hypohidrosis, increased sweat chloride; angiokeratomas; vacuolated lymphocytes; glycolipid storage and oligosaccharide and glycopeptide excretion; and defective activity of α-fucosidase.

INTRODUCTION

Fucose is a deoxysugar, an aldohexose in which the terminal CH_2OH is replaced by a methyl group (Figure 95.1). It occurs in glycoproteins and glycolipids as a terminal oligosaccharide linked to galactose or N-acetylglucosamine (Figure 95.2). The degradation of glycoproteins takes place sequentially in the lysosomes. Fucosidosis is a glycoprotein storage disease in which patients have impaired degradation of fucose-containing glycoproteins.

Fucosidosis was described first in 1968 by Durand and colleagues in two brothers [1, 2]. The enzyme defect was reported in the same year by Van Hoof and Hers [3]. Heterogeneity was recognized early. Most patients encountered, have had the fatal infantile form of fucosidosis, but more indolent phenotypes have been reported with survival even to adulthood [4, 5]. There has been a tendency to classify these variants as type II [6] or III, with the infantile as I, but it is increasingly clear that a spectrum of mutation leads to a spectrum of variability in clinical expression [7], and some patients with the same mutation have had very different phenotypes. The gene (*FUCA1*) has been mapped to chromosome 1 p36 [8, 9]. A number and variety of mutations have been identified. One mutation that causes a premature termination (Q422X) was found in eight families [10, 11], but most mutations have been unique to a single family [12, 13].

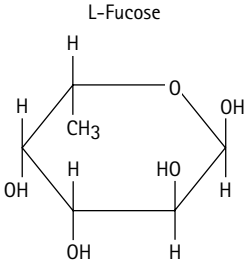

Figure 95.1 L-Fucose.

CLINICAL ABNORMALITIES

The classic infantile phenotype (Figures 95.3 and 95.4) is Hurler-like; in that patients appear normal at birth, but during late infancy they develop progressive coarsening of the features, and impaired linear growth and cognitive

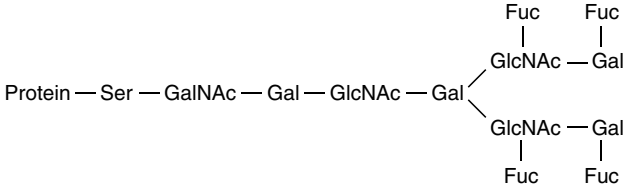

Figure 95.2 Glycoprotein structure with terminal fucose residues. The N-glycosidic linkage to amino acid could also be to threonine. In addition to the linkage shown there is N-glycosidic linkage to the asparagine residues of proteins by GalNAc. Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; Ser, serine.

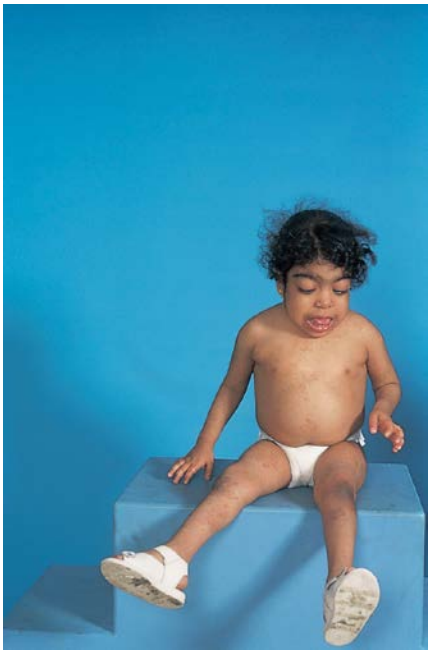

Figure 95.3 A five-year-old Saudi girl with fucosidosis. She was mentally impaired and short and had coarse features. The cornea was hazy. Two siblings were affected.

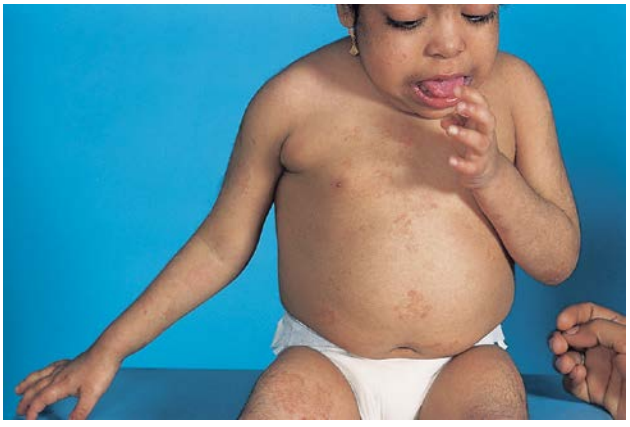

Figure 95.4 The same patient. Her abdomen was protuberant. The tongue was large. α-Fucosidase activity of cultured fibroblasts was 0.05 percent of control.

development. Cerebral degeneration and mental deterioration progress to dementia and spasticity. There is gradual loss of muscle strength and tremor. The protuberant abdomen is a consequence of hepatosplenomegaly. The cornea may be hazy. Roentgenograms reveal the typical appearance of dysostosis multiplex (Chapter 76). Imaging of the central nervous system may reveal atrophy, hypomyelination, and increased T2 intensity in the cerebral white matter. The concentration of chloride in the sweat is quite high. Respiratory infection may be a problem. An end-stage decerebrate rigidity is usually followed by death within the first decade.

In the more indolent disease [7, 14] the first sign may be the development of angiokeratomas (Figure 95.5), which

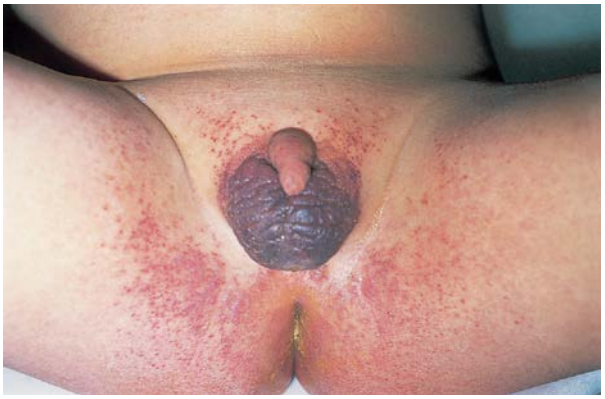

Figure 95.5 Angiokeratomas may be prominent, as in the inguinal area of this patient. (The illustration was kindly provided by Dr John Aase of Albuquerque, New Mexico.)

may be present as early as six months to four years. By 20 years of age they are seen in 85 percent of patients [7]. They are prominent over the buttocks and genitalia and are indistinguishable from those of Fabry disease (Chapter 87). Red streaks may be noted on the gingivae even earlier, and may be perpendicular to the roots of the teeth. There may be tortuosity of conjunctival vessels [15]. Pigmentary retinopathy has been observed [15]. The skin may appear thickened or dry and thin [6]. With time, facial features become coarse, and the eyelids may be puffy. These patients may have normal sweat chloride concentrations, but they may have hypohidrosis, or difficulty controlling body temperature [6]. Hepatosplenomegaly is not characteristic. Mental deterioration is slower, and patients may live to adult life. Neurologic features include a stiff broad-based gait, spasticity, increased deep tendon reflexes, and positive Babinski responses. Some patients have seizures. One patient had rapidly progressive dystonia [16]. Hearing loss has been observed [7]. Stature is reduced, but head circumference is normal [7]. The skeletal abnormalities are those of a dysostosis multiplex, which may be milder [14, 17]. The spine, pelvis, and hips may be the most affected. Vertebral bodies are flattened and beaked, and there may be odontoid hypoplasia. There may be clinical kyphoscoliosis. Coxa valga is associated with flattening of the femoral heads and widened, scalloped, sclerotic acetabula. Shafts of the long bones may be wide. Neuroimaging reveals changes in the thalamus, globus pallidus, and internal capsule [18, 19].

Another phenotype [20] in which α-fucosidase was deficient was that of spondyloepiphyseal metaphyseal dysplasia. Stature was quite short, but mental development was normal. Problems in classification are highlighted by the occurrence of mild and severe presentations in the same sibship [21] and among patients homozygous for the common 422-stop mutation [7]. In addition, a patient with an initial mild appearance went on to a rapidly fatal progression [22]. Another patient with late onset of neurologic features had dry skin and blue-brown spots on the tongue and sclerae [23], as well as macroglossia. This patient had MRI findings of abnormal pallidal signaling interpreted as "eye of the tiger".

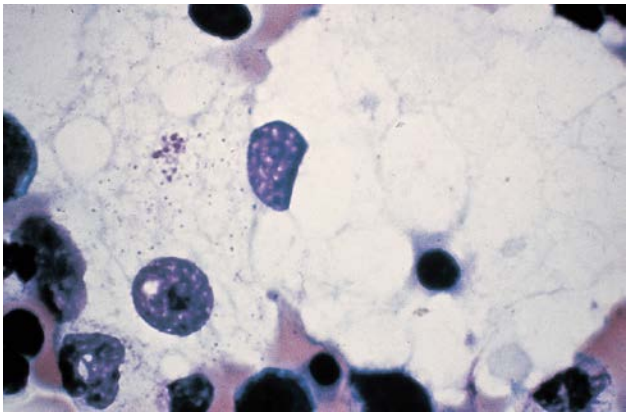

Figure 95.6 Foam cell in the marrow of a patient with fucosidosis.

Vacuolated lymphocytes are visible in the peripheral blood, and histologic examination of the liver reveals foamy cytoplasm and vacuoles, some with lamellar structure [24, 25]. Foam cells are demonstrable in the bone marrow (Figure 95.6). Vacuoles may also be demonstrable in the sweat glands on skin biopsy [14], or in conjunctival cells [26]. Abnormalities have been reported in fibroblasts and in Schwann cells visualized after rectal biopsy [27]. The brain was large at autopsy and the adrenals atrophic [28]. Storage vacuoles have also been found in the ultrastructure of the brain [24].

GENETICS AND PATHOGENESIS

Fucosidosis is autosomal recessive. Consanguinity has commonly been noted [23]. The gene for the infantile type is common in Reggio Calabria in southern Italy [29]. In the US, the disease has been found in the southwest (Figure 95.5). Approximately 100 patients have been reported [30].

The gene is composed of eight exons spanning 23 kb [31]. Three patterns of mRNA were found [32] in Italian patients: two lacked mRNA; one had reduced amounts of an RNA with a cDNA by hybridization of a pattern indicating loss of a restriction site; and three had mRNA that was normal in size and content. Among mutations defined, a deletion of two exons [10] resulted in marked reduction of cross-reacting material (CRM) and absence of enzyme activity; a C-to-T transition leading to a TAA stop-codon, p.Q422X, deleting the carboxyl end of the enzyme [16, 33, 34]. This mutation causes loss of an EcoRI restriction site that is useful for molecular diagnosis [11]. A C-to-A change in exon 6 led to a stop codon p.W382X [12]. Among other mutations, most have been missense, not deletions or other major changes in gene structure [10–13, 35, 36]. Nonsense mutations were common. A homozygous nonsense mutation c1295G>A,p.W423X was found in exon 8 in a patient with unusual phenotype [23\ (v.s.). A patient with a missense mutation, p.L405R, was 46 years old despite less than 1 percent of control enzyme activity and no CRM [37]. Most of the mutations reported have led to virtual absence of activity of the enzyme, and this has been independent of the variability seen clinically.

Defective activity of the enzyme can be demonstrated in leukocytes and cultured fibroblasts [14, 38, 39]. Routine assays use artificial substrates and fluorimetric or colorimetric analysis. The different phenotypes cannot be distinguished by enzymatic assay as activity is essentially absent in all. There is fucosidase activity in serum or plasma, but assay is not a reliable method of diagnosis, as some normal individuals have low levels of activity in the fluid [40]. Heterogeneity among patients has been shown by the assessment of amounts of enzyme protein [41]; of 11 patients with markedly defective enzyme activity, eight made no enzyme protein in fibroblasts; in two, the amounts of the 53 kDa precursor were normal, but there were no mature 50 kDa form; in one, there was a small amount of CRM.

Heterozygotes tend to have activity values intermediate between patients and controls in leukocytes or fibroblasts [14, 39, 42], but there is sufficient overlap with controls that heterozygote detection and screening for carriers in a high-risk population are not reliable. Prenatal diagnosis has been accomplished [42] by assay of the enzyme in cultured amniocytes. In families in which the mutation is known, this is the method of choice for prenatal diagnosis and heterozygote detection [7].

Complementation analysis of cells from patients with the different phenotypes did not yield restoration of activity [39].

A variety of fucose-containing glycolipids and glycoproteins accumulate in patients with fucosidosis. The blood groups H and Lewis are degraded with difficulty and may be present in high concentration. The H antigen glycolipid, Fuc-Gal-GlcNAc-Gal-ceramide, accumulates [43]. A variety of oligosaccharides are found in the urine [44], and this provides an approach to the initial diagnosis, although most request enzymatic analysis in a patient in whom the diagnosis is suspected. Thin layer chromatography and staining with orcinol gives a diagnostic pattern in fucosidosis, mannosidosis, sialidosis, and aspartylglycosaminuria. The glycopeptides found in the urine in fucosidosis all have GlcNAc linked to asparagine [45], often with fucose in a-1,6 linkage with GlcNac. In addition, the fucosylGlcNAc disaccharide is found. Thin layer chromatography of the urine and staining with ninhydrin, followed by heating to 120°C, yields a bright blue spot in fucosidosis (as in aspartylglycosaminuria) that may be useful in screening [46].

TREATMENT

Only supportive treatment is available. In canine fucosidosis in Springer spaniels, bone marrow transplantation led to increased enzyme activity in neural as well as visceral tissues and reduction of storage along with clinical amelioration [47, 48]. Bone marrow transplantation in an eight-month-old patient with fucosidosis yielded a much milder degree of developmental delay 18 months later than observed in his affected sibling at the same age [49].

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96

α-Mannosidosis (β-Mannosidosis)

MAJOR PHENOTYPIC EXPRESSION

Severe mental and motor retardation with deterioration and early death; coarse features; hepatosplenomegaly; dysostosis multiplex; cataracts and corneal opacities; deafness, deficient immune function, storage of mannosylglycoproteins, urinary excretion of mannosyl-oligosaccharides and defective activity of α-mannosidase, and mutations in the *MAN2B1* gene.

INTRODUCTION

Patients have been increasingly recognized in which the clinical features were those of mucopolysaccharidosis but there was no mucopolysaccharidiuria. The recognition of inclusions set out I-cell disease (Chapter 83) as a distinct entity in 1967. In the same year, Öckerman [1] described α-mannosidosis. The enzyme (Figure 96.1) exists in at least two forms, which are immunologically indistinguishable and are coded for by a single gene, *MAN2B1*, on chromosome 19 p13–q12 [2]. The gene has been sequenced [3–5]. A mutation, 212A>T, was found in two siblings of a consanguineous mating [6]. Other mutations in a full spectrum of mutation types have been reported [5, 7, 8]. A total of 126 disease causing mutations has been reported [5].

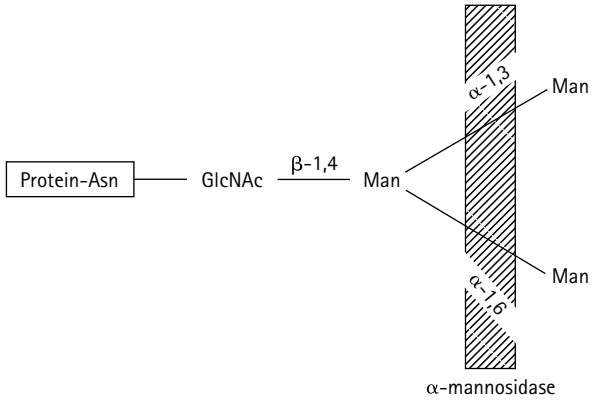

Figure 96.1 The mannosidase reaction, Asn, asparagine; GlcNAc, N-acetylglucosamine; Man, mannose.

CLINICAL ABNORMALITIES

Clinical features in more than 90 patients with the disease have reflected considerable phenotypic diversity [2, 9–17]. Patients have been classified into the severe infantile type of disease and a more indolent form. It is already clear that there is a spectrum including a wide variety of expression. Phenotypic heterogeneity within sibships has also been described [18], which means that there are modifiers of expression, and that classification into two forms is simplistic.

The infantile or classic form of mannosidosis is characterized by a very early onset of a disease that resembles a severe mucopolysaccharidosis (Figures 96.2–96.5). Hernias may be among the earliest findings. Facial features are very coarse. Teeth may be widely separated. The skin may feel thickened, indicating the presence of stored material. Hepatosplenomegaly is prominent. There may be noisy breathing, nasal discharge, or frequent respiratory infections. Macrocephaly is present, along with frontal bossing [13]. Mental development is severely retarded. Speech development may be worse – a consequence of impaired hearing. Gait may be broad-based.

Dysostosis multiplex is extreme [19]. The head may be large and the forehead prominent. The sella turcica is J-shaped and the calvaria thickened. Vertebral bodies are

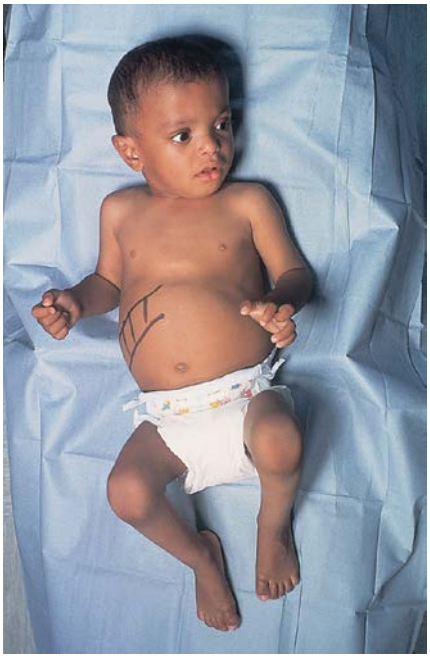

Figure 96.2 AMR. A 20-month-old Saudi Arabian boy with mannosidosis. At this age, he could stand and cruise, but did not walk or speak. The activity of a-mannosidase in fibroblasts was 10 percent of control.

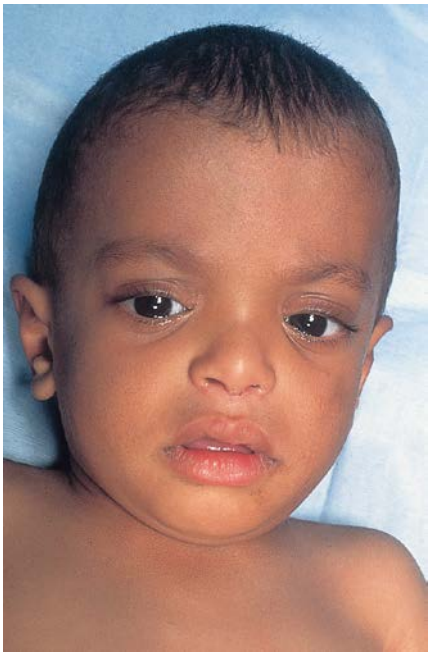

Figure 96.3 AMR. The facies of this patient, especially the lips and nose, suggested the presence of storage material.

hypoplastic and flattened or ovoid with anterior beaking. A gibbus may be present. Proximal metacarpals are tapered and the iliac wings flared. Pulmonary infiltrates are commonly seen. There may be corneal opacities and posterior lenticular cataracts in a spoke-like pattern [20–22].

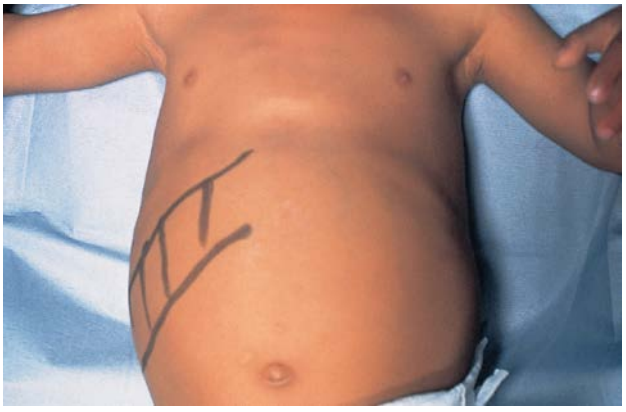

Figure 96.4 AMR. The abdomen was protuberant. The hepatomegaly is outlined. The spleen was also enlarged. Bone marrow revealed foamy histiocytes.

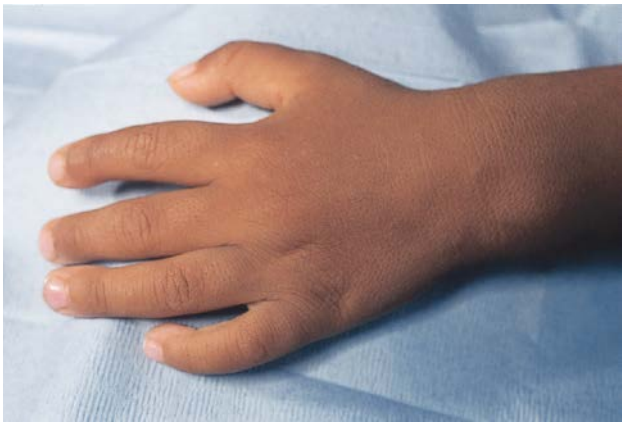

Figure 96.5 AMR. He had clinodactyly of the fifth fingers and proximally placed thumbs.

Deterioration may be rapid, and most patients die between three and 10 years of age, often of pneumonia.

Other patients may have a more indolent course. A majority of patients have had a moderate form of disease, sometimes classified as type 2 [23].

Major features are mental retardation and hearing loss [24–26] (Figures 96.6 and 96.7). Survival into adulthood is common. Some of these patients have mild dysostosis multiplex, while others do not [24]. Other skeletal problems include kyphoscoliosis (Figure 96.6) and destructive synovitis of the knees [26, 27]. Hepatosplenomegaly may be absent. The eyes are usually clear, but retinal degeneration has been reported [7] including the appearance of yellow white deposits. Hearing loss is progressive, and storage material has been found in the ear (Figure 96.7).

Hydrocephalus has been reported [28] and spastic paraplegia [29], as well as ataxia [7]. Progressive cerebellar ataxia of late adolescent onset was the initial clinical manifestation is three adult siblings [30]. They also displayed nystagmus, dysarthria, and positive Babinski responses. Hyperphagia has been observed [17].

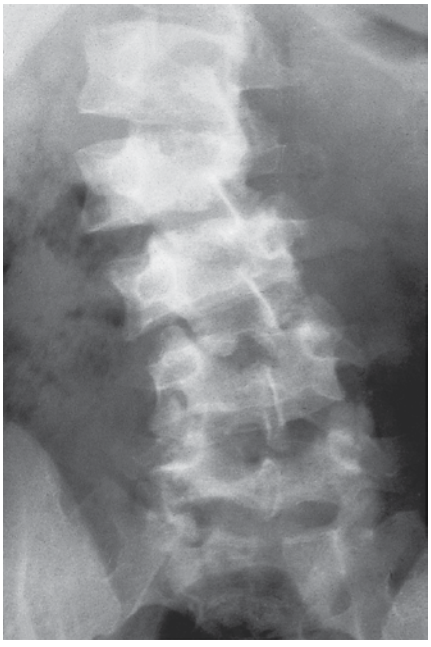

Figure 96.6 Roentgenogram of the spine of patient with mannosidosis shows scoliosis. There was generalized dysostosis multiplex. (Illustration was kindly provided by Dr Philip Benson.)

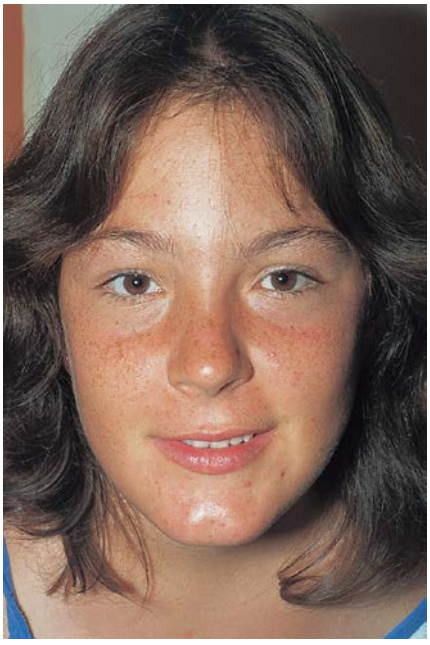

Figure 96.7 DF. A 15-year-old with a mild variant of mannosidosis. She had a learning disability from infancy and had an IQ of 78. She had bilateral hearing loss. Surgery on the right middle ear at 19 years revealed extensive deposits of mannoside.

Cardiovascular abnormality has been manifested by premature ventricular contractions and a shortened PR interval on electrocardiogram (EKG) [31]. Magnetic resonance imaging (MRI) findings have included cerebellar atrophy and abnormal signal in the white matter. A patient with delusions and hallucinations had cerebellar atrophy and periventricular white matter changes on MRI [32]. In a series of 35 patients, [33] with a milder type 2 phenotype, all were socially dependent and unable to care for themselves. Regardless of the phenotype degeneration with time appears to be the rule.

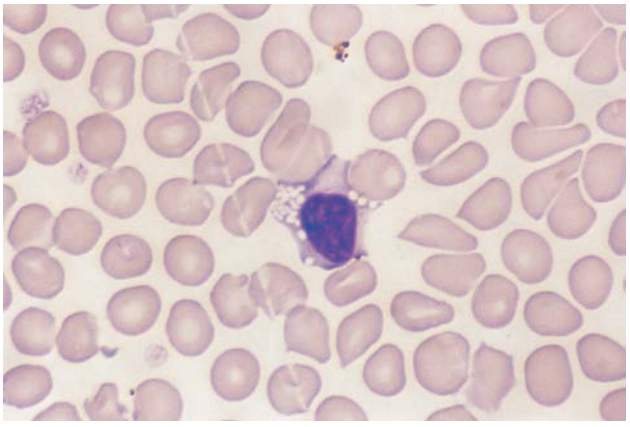

Figure 96.8 Vacuolated lymphocytes from peripheral blood.

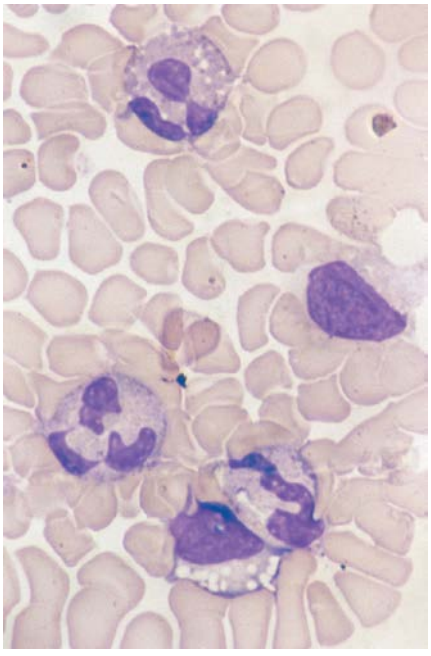

Figure 96.9 Polymorphonuclear leukocytes were also vacuolated.

Hematologic evaluation regularly reveals vacuolated lymphocytes in patients with all types of mannosidosis [13, 24] (Figure 96.8). Polymorphonuclear leukocytes may also be vacuolated (Figure 96.9), and the bone marrow may reveal foamy macrophages. Pancytopenia has been reported [34]. Increased susceptibility to infections, both bacterial and viral including bronchitis and otitis media has been documented and associated with a variety of abnormalities in leukocyte function [13, 35], including defective response to chemotactic stimulation and slowed phagocytosis. The ability of lymphocytes to undergo transformation was reduced. There may be some reduction in IgG.

Histopathology has revealed foamy, vacuolated hepatocytes [9, 13, 28, 29]. Vacuoles have also been observed in histiocytes, lymphocytes, [16] muscle and fibroblasts [7]. Neuronal changes were widespread in the central nervous system [13, 36], with ballooning and ultrastructural evidence of storage vacuoles.

GENETICS AND PATHOGENESIS

α-Mannosidosis is an autosomal recessive disease, and affected offspring have been of both sexes [8]. The gene has been assigned to chromosome 9 [36] to the central region between p13.2 and q12 [2, 37]. It contains 24 exons. The mutation p.R750W has accounted for 27 percent of disease producing alleles in 16 countries [5, 38]. Eighty-three novel mutations were identified extending the mutant spectrum to 125. The mutation at nucleotide 212 leads to the H71L amino acid change [6]. Two other mutations were reported [39] in two homozygous Italian patients with α-mannosidosis, IVS-2A>G and 322-323insA. The first led to skipping of exon 21. The second caused a frame shift with a stop codon at amino acid 160. A 47-year-old Japanese woman with a homozygous C>T change in exon 19 leading to R760X had less than 1 percent of 99 control enzyme activities [7]. Q639X and R750W were found in a 7-year-old Finnish boy [7].

The defective enzyme, acid α-mannosidase (Figure 96.1), is lysosomal and is synthesized in a precursor form followed by processing into smaller subunits assembled in human liver into forms which are separable by chromatography and electrophoresis but immunologically indistinguishable [8, 13, 40–42]. Residual activity in affected patients usually ranges between three and five percent of control [15, 25, 43]. Some variant patients have had higher (15–20 percent) residual activity [25, 43]. Levels in leukocytes tend to be lower than those in fibroblasts, but the diagnosis can be made with either. The diagnosis has also been evident on assay of the enzyme in plasma, but this is not recommended as reliable [13]. Immunologic cross-reactive material appears to be present in most patients [15, 43].

Prenatal diagnosis has been carried out by assay of the enzyme in cultured amniocytes or in chorionic villus material [44–49]. Normal activity in chorionic villi may be considerably less than in amniocytes [47]. Accurate prenatal diagnosis must take into account not only the issue of variant residual activity, but forms of α-mannosidase that are not defective in patients with mannosidosis [41].

Heterozygotes may have intermediate levels of enzyme activity, but they are more often normal [13, 15]; therefore, this is not reliable. If the mutation is known, molecular analysis is the method of choice for both prenatal and heterozygote detection.

The result of the defective enzymatic activity is the storage of a variety of glycoproteins and glycoprotein-derived oligosaccharides. These have been best characterized

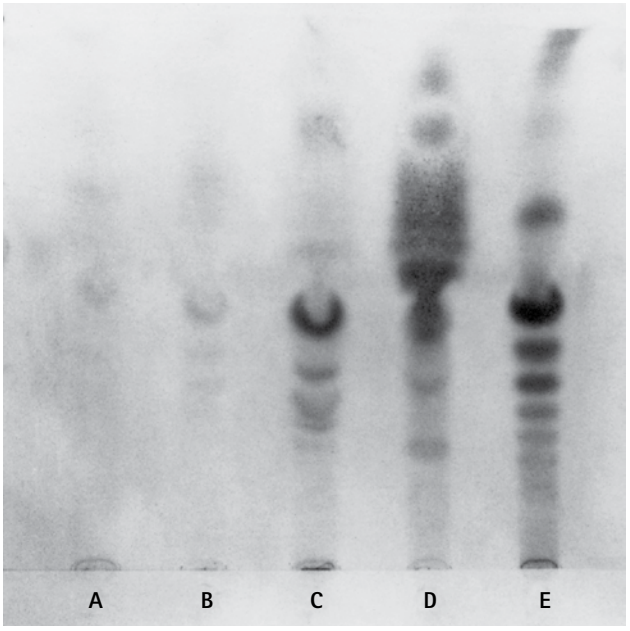

Figure 96.10 Thin layer chromatogram of urinary oligosaccharides. Lane A was normal urine: lane B the patient, and lane C a patient with classic mannosidosis. Lane D and E were the normal and patient 20x concentrated, indicating the presence in DF of smaller concentrations of the oligosaccharides than seen in unconcentrated urine in the more classic mannosidosis phenotype. (This illustration and Figure 96.11 were kindly provided by Dr Thomas G Warner and are printed with permission from *Clin Genet* 1984;**25**:248.)

in the urine [48–50], and it is by study of the patterns of urinary oligosaccharides (Figures 96.10 and 96.11) that the diagnosis has usually been first made chemically, mainly by thin layer chromatography (Figure 96.10) [15, 24, 51, 52]. There are a number of mannosyloligosaccharides in the urine of these patients. The major one is the trisaccharide, Man-α (1,3) Man-β(1,4) GlcNAc [24, 53, 54] (Figure 96.11).

Affected patients, and those with aspartylglucosaminuria, have been reported to have elevated levels of dolichol in the serum [55, 56]. This could prove useful in diagnosis. It may reflect the fact that complex glycoproteins are synthesized by the transfer of oligosaccharide precursor from dolichol to the asparagine of the peptide.

TREATMENT

Bone marrow transplantation has been reported [57] to stabilize intellectual function and improve hearing.

β-Mannosidosis

Another disorder of glycoprotein catabolism, β-mannosidosis, was first described in goats who displayed a severe degree of neurodegeneration [58]. They had defective activity of β-mannosidase.

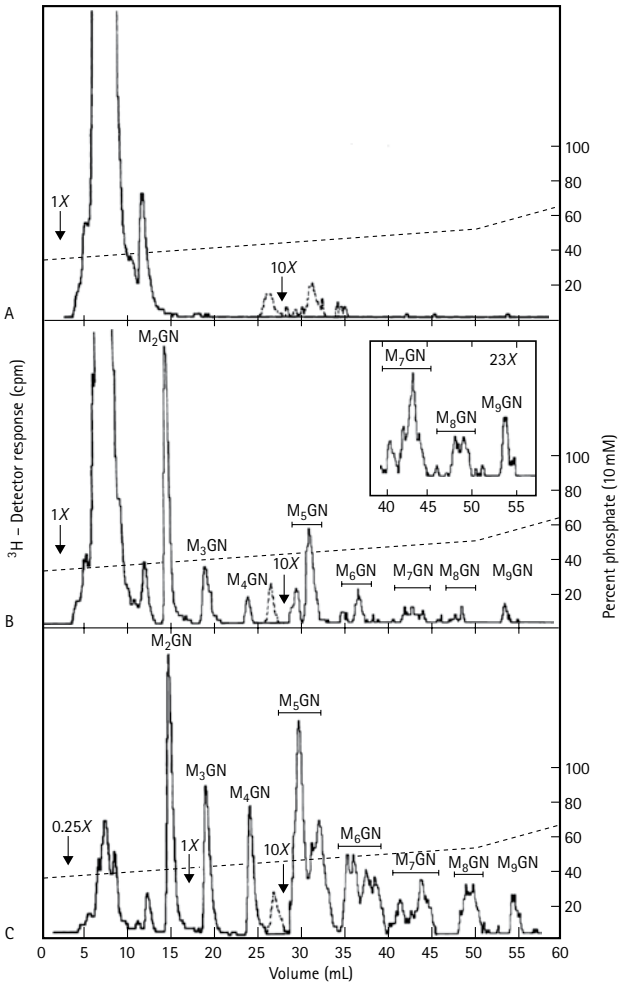

Figure 96.11 HPLC chromatogram of urinary oligosaccharides as 3H-aldols. A was normal urine, B the patient and C half as much urine of the classic patient. The mannose containing fractions were labeled M, e.g. M_2GN, the major fraction, was the trisaccharide Man-α (1-3) Man-β (1-4)GlcNAc.

It is now apparent that this disease also occurs in man. Severity of disease is variable but may be mild [59]. An example was a 44-year-old man with impaired mental development and no other neurologic abnormalities, but he had scrotal and penile angiokeratomas [60]. Deafness and speech retardation were reported in two consanguineous Turkish siblings [61]. An 18-year-old patient with bilateral hearing loss and mild cognitive impairment also had symptoms of Tourette syndrome [62]. A different spectrum was reported [63] in a patient with progressive spinocerebellar ataxia and a novel mutation. In another patient [64], there were neonatal seizures and communicating hydrocephalus.

Mutations in the *MANBA* gene have been found in many of these families, many of them null mutations [59, 62, 65], such as E83X, Q426X, and 1541 in patients with a range of severity from mild to severe [59]. The disaccharide stored is one of the smallest observed in lysosomal storage disease.

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97

Galactosialidosis

MAJOR PHENOTYPIC EXPRESSION

Early infantile
Edema, fetal (hydrops fetalis)-neonatal; telangiectases; hepatosplenomegaly; growth failure; psychomotor delay and deterioration; dystosis multiplex; cardiac failure; proteinuria; and death in infancy.

Late infantile
Hepatosplenomegaly; dystosis multiplex; corneal clouding; hernias; valvular cardiac disease; shortness of stature and hearing loss.

Juvenile (adult)
Ataxia; myoclonus; seizures; impaired mental development and deterioration; corneal clouding; angiokeratomas; platyspondyly; and shortness of stature.

Each type
Coarse facies; cherry red spots; foam cells; defective activity of β-galactosidase and neuraminidase resulting from mutations in the gene for the lysosomal protective protein/cathepsin A (PPCA).

INTRODUCTION

Following the discovery in 1968 of β-galactosidase deficiency in generalized GM_1 gangliosidosis [1], a number of patients were reported with atypical features such as cherry red macular spots and an absence of hepatosplenomegaly [2–5]. The combination of features of cerebral lipidosis and mucopolysaccharidosis without mucopolysaccharidurie suggested a mucolipidosis. Complementation studies in somatic cell hybrids indicated that two of these patients [4, 5] had mutations distinct from or non-allelic with GM_1 gangliosidosis, though they were clearly deficient in β-galactosidase [6]. Wenger *et al.* [7] in 1978 reported the coexisting deficiency of neuraminidase and β-galactosidase in leukocytes and fibroblasts of a patient with what had been thought [8] to be a variant form of GM_1 gangliosidosis. Then other patients were reported in whom sialidase deficiency was present [9–12] along with that of β-galactosidase.

That the primary defect was not in sialidase was shown by complementation of the cells of patients with sialidosis by hybridization with cells of patients with the combined defect [11, 13]. Further, in the combined defect cells, both enzyme defects could be restored by a glycoprotein corrective factor produced in culture by normal fibroblasts or those of β-galactosidase deficiency indicating the presence of a third protein acting as a corrective factor. The turnover of β-galactosidase in normal fibroblasts was 10 days, while in the cells deficient in both enzymes it was less than one day [14] and, in experiments with purified enzyme, it was clear that the rapid turnover was caused by proteolytic degradation of the enzyme [15, 16]. The disorder was named galactosialidosis in 1981 [17]. The molecular defect was found by d'Azzo and colleagues [16] to be in the PPCA, which aggregates with both enzymes to form multimers that resist lysosomal degradation. The gene has been mapped to chromosome 20q13.1 [18, 19],

and the human cDNA has been cloned [20]. Mutations have been discovered [21, 22]. The number of patients reported has been small, the majority in the juvenile or adult variants.

Multienzyme complexes may work synergistically and provide more efficient responses to changes in the load and composition of substrates. The glycoprotein storage diseases, β-galactosidase deficiency, neuraminidase deficiency, and PPCA, the defect in galactosialidosis, are representative of such a multiprotein complex [23]. In contrast, GM_1 gangliosidosis is a glycosphingolipids storage disease. Both are components of the lysosomal network of organelles involved in sorting, digestion, recycling, and secretion of cellular components. The functional mutations in each component of the glycoprotein complex result in impressive lysosomal storage disorders. The multiprotein complex includes the protective cathepsin A protein in which mutations lead to galactosialidosis. The protective protein protects both enzymes from proteolytic degradation. Mutations have been reported in the PPCA gene [24]. Features of GM_1 gangliosidosis and sialidosis result from defective activity of both β-galactosidase and neuraminidase as a result of fundamental deficiency in the lysosomal PPCA, which is itself a serine carboxypeptidase.

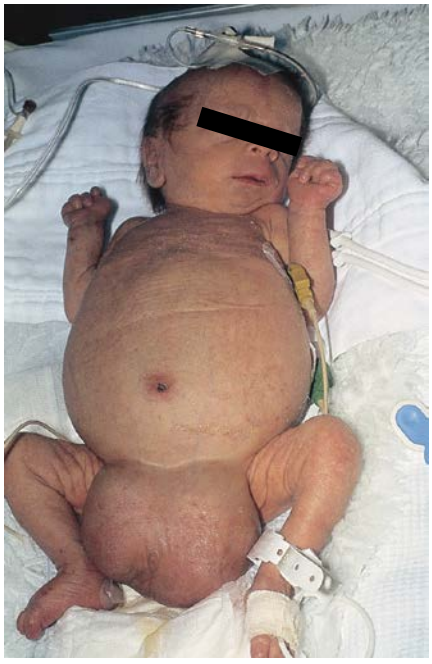

Figure 97.1 A newborn infant with galactosialidosis who presented with non-immune hydrops fetalis and had extreme edema of the vulva.

CLINICAL ABNORMALITIES

Considerable phenotypic heterogeneity has been observed consistent with different mutations. Nevertheless, it continues to appear useful to distinguish the early infantile and late infantile phenotypes, while the rest, accounting for 70 percent of the patients, have been called the juvenile/adult type and appear to represent quite a broad spectrum of variants.

The early infantile is the most severe form of the disease. Fetal hydrops may lead to stillbirth or early neonatal death [24]; extensive edema may be evident in the neonatal period (Figures 97.1–97.3). Features are coarse [25–30] (Figures 97.4 and 97.5), and there is hepatosplenomegaly. Inguinal hernias are common. Psychomotor delay may be global, and deterioration is progressive to death at an average age of seven months. Dysostosis multiplex is uniformly present; it may be less prominent than in other forms of dysostosis because of the short interval in which to develop before demise. Telangiectases have been found in the early infantile disease, but angiokeratomas are rarely seen. There may be corneal clouding and cherry red spots [25]. Proteinuria is an early sign of renal dysfunction; renal failure may ensue [29]. Infiltration of the heart leads to thickened septa, cardiomegaly, and congestive failure may occur as early as the first week of life [25–27, 31]. Recurrent fetal hydrops has been reported in two families [32, 33]. Thrombocytopenia with purpura and anemia were reported in a patient with fetal hydrops [34]. Anemia and thrombocytopenia were also found, along with hemophagocytosis in a seven-month-old boy [35]. In an early infantile patient with fetal hydrops,

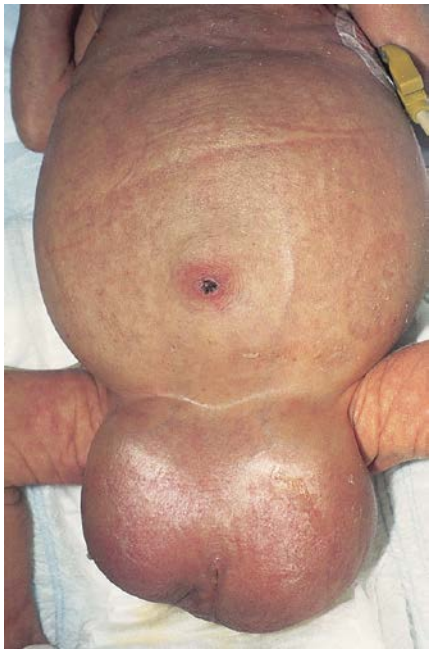

Figure 97.2 Close-up of the edematous genitalia. A mutation V132M was found by analysis of the DNA by Dr Suzuki.

there were massive fluid filled inguinal hernias, multiple telangiectasia and diffuse hypopigmentation [36]. Punctate stippled epiphyses were found in femora, calcanei, and sacrum [36].

The late infantile form of the disease may be evident as early as the first month of life. Patients have coarse features, hepatosplenomegaly, and dysostosis multiplex

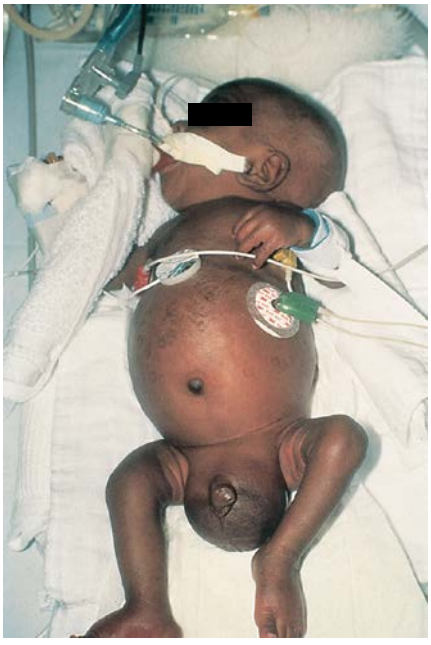

Figure 97.3 Another infant with hydrops fetalis.

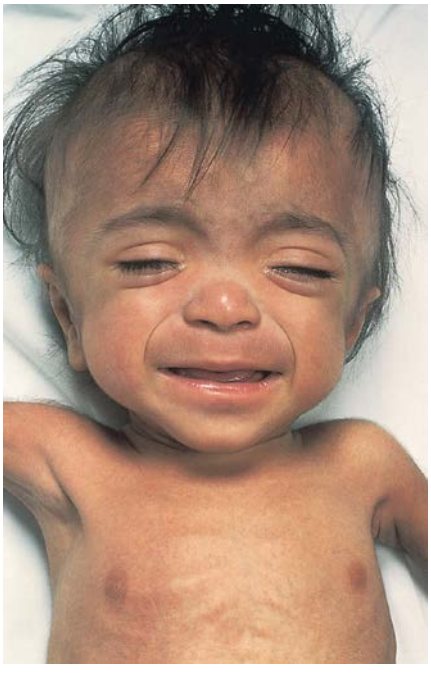

Figure 97.4 A seven-month-old Omani child with galactosialidosis. The features were quite coarse and the eyebrows abundant. There was a substantial amount of hair on the head despite almost complete absence of subcutaneous tissue. There were cherry red macular spots.

with the appearance of mucopolysaccharidosis [5, 17, 37–39]. Some have had cherry red spots and/or corneal clouding [17, 38]; others have not [5, 37]. Generalized seizures or petit mal has rarely been observed [5]. Impaired mental development in these patients has generally been mild. Neurologic deterioration has not generally been seen [5].

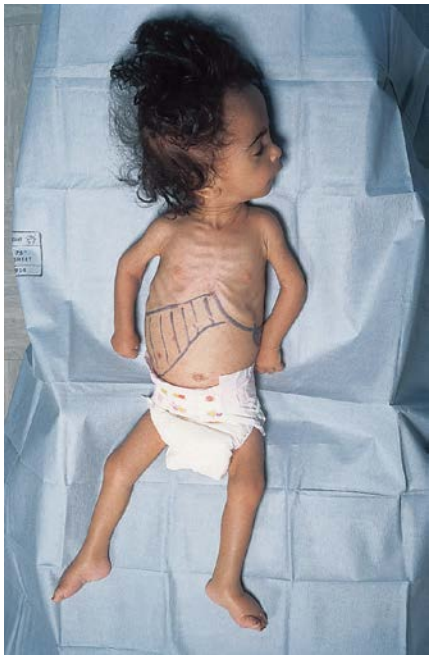

Figure 97.5 A one-year-old with galactosialidosis. Impaired development was global. The liver and spleen were enlarged, and there were cherry red macular spots.

Cardiac involvement is a regular feature of the disease. Valvular involvement has included thickened mitral and aortic valves. Aortic valve replacement has been required by 16 years of age [37]. Hearing loss may be conductive or mixed. Shortness of stature may be a consequence of disease of the spine, and there may be atrophy of the muscles. Angiokeratomas are uncommon.

Patients classified as juvenile/adult have varied considerably in severity. A sizable number has been reported from Japan [12, 13, 29, 39–50]. In a Mexican family with first cousin parents, two boys and a girl had coarse features, dysostosis multiplex, shortness of stature, and impaired mental development, along with cherry red spots, corneal clouding, seizures, and hearing loss, but no hepatosplenomegaly [2]. Onset of symptoms has been as early as one year of age [45] or as late as 40 years [29]. Coarse features are regularly seen, but they may be mild (Figure 97.6). Most patients have platyspondyly, but fully developed dysostosis multiplex is unusual. Hepatosplenomegaly is not common. Novel findings include tracheal stenosis and AV nodal re-entry trachycardia.

Neurologic features include generalized seizures and myoclonus, ataxia, and impaired mental development. Deterioration may be progressive. Deep tendon reflexes are brisk [26]. Bilateral cherry red spots are found in most patients (Figure 97.7). There may be corneal clouding, punctate lenticular opacities, and loss of visual acuity. Other patients have no neurologic abnormalities.

Angiokeratomas are common [29, 46] (Figure 97.8). They are found in clusters in a distribution indistinguishable from those of Fabry disease (Chapter 87).

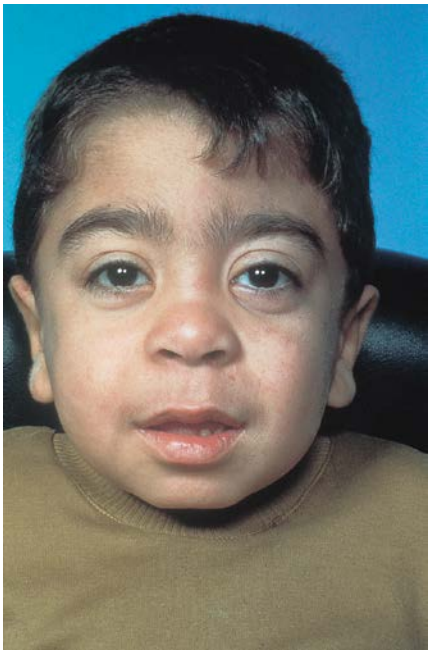

Figure 97.6 A 5-year-old boy with galactosialidosis. He had mildly impaired mental development and had mildly coarse features and hirsutism. Dr Suzuki found a seven-nucleotide insertion between exons 13 and 14.

All patients with galactosialidosis have foam cells in the marrow and vacuolated lymphocytes in peripheral blood. Vacuoles may also be seen in Kupffer cells [51]. Pathologic features include macroscopic cerebral atrophy [51]. Membrane-limited vacuoles are seen on electron microscopy of lymphocytes or skin [38], brain [54], endothelial cells [55] peripheral Schwann cells [55, 56],

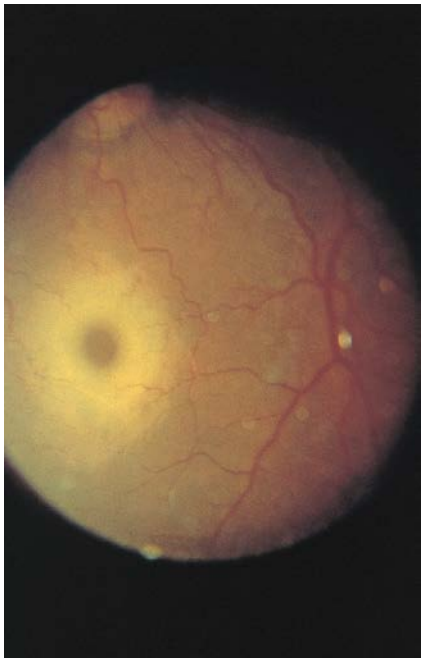

Figure 97.7 The cherry red spot of the five-year-old boy.

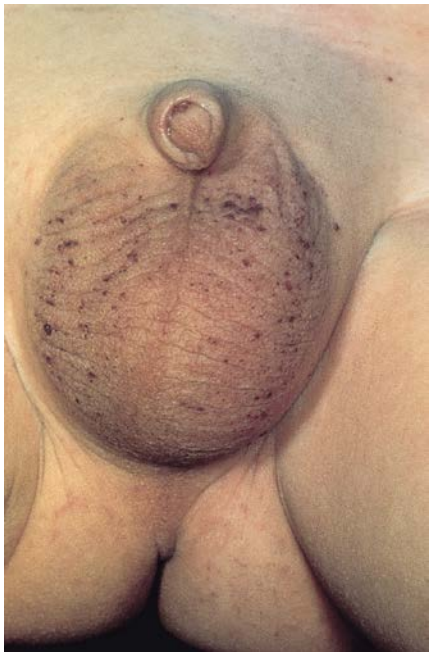

Figure 97.8 The five-year-old had angiokeratoma of the scrotum. Histologic analysis was confirmatory.

and in the myenteric plexus of the rectum [44, 49]. Their appearance is similar to those of GM_1 gangliosidosis and sialidosis. They may have lamellar or wavy concentric structure [52, 53]. An early infantile patient was reported in which multiple infarctions were found in the brain [57]. In another, there were periventricular calcifications [30].

GENETICS AND PATHOGENESIS

The disorder, in all of its variants, is transmitted in an autosomal recessive fashion. Rates of consanguinity have been quite high. Fibroblasts of a parent were shown to have reduced amounts of mRNA for the protective protein, providing chemical evidence of heterozygosity [58]. Enzyme activity attributable to the protective protein also yielded intermediate values in fibroblasts of heterozygotes [59, 60].

The molecular defect is in the lysosomal PPCA, the existence of which became evident through studies of patients with galactosialidosis [18, 61]. This protein is normally synthesized as a 54 kDa precursor, which is modified post-translationally to 32 and 20 kDa polypeptides which proved to be the corrective factor [13, 16, 62]. Immunoprecipitation demonstrated absence of the 54, 32, and 20 kDa polypeptides in fibroblasts of patients with galactosialidosis [16]. Neuraminidase aggregates normally with β-galactosidase and protective factor in a large multimer that resists proteolytic degradation [62–65]. Neuraminidase requires protective protein for activity. The multimer aggregate correctly routes the two glycosidases to the lysosome and protects them from rapid lysosomal proteolysis. The isolation of the cDNA for the protective

protein [58] and its expression in COS cells [59, 65] elucidated the structure, function, and physiology of the protein. The sequence begins with a signal peptide that is cleaved, followed by 298 amino acids of the 32 kDa protein, which is followed by the 20 kDa protein; the two make up the 54 kDa precursor. The latter, synthesized in COS cells from the cDNA, is taken up by the fibroblasts of patients with galactosialidosis and restores activity in both enzymes [58].

Once the primary structure of the protein was known, its homology to yeast and plant serine carboxypeptidases became apparent [66]. The protective protein was then shown to have carboxypeptidase activity [67], and this activity is deficient in galactosialidosis. The properties of this carboxypeptidase are consistent with those of cathepsin A [59]. Site-directed mutagenesis which abolishes cathepsin activity does not interfere with protective activity; so, the two functions are distinct [59]. Nevertheless, cathepsin A activity provides a simple test that is useful for heterozygote detection. The fact that the protective function and cathepsin activity are distinct provides an argument for continued diagnostic reliance on the assay of β-galactosidase and neuraminidase in leukocytes or fibroblasts [58, 59]. The three enzyme activities, cathepsin, β-galactosidase, and neuraminidase, copurify [58]. PPCA and galactosidase are found separate from the complex, but all of the neuraminidase is present in the complex [68]. PPCA functions as an intracellular transport protein [69]. It has a mannose-6-phosphate recognition marker [70]. The enzyme also has deamidase and esterase activities, and these activities are deficient in cultured cells of patients [71].

Prenatal diagnosis of an affected fetus has been accomplished by assay of β-galactosidase and neurominidase in cultured amniocytes [32] and also by the detection of sialyloligosaccharides in amniotic fluid [72]. In a patient with intrauterine poor growth and oligohydramnios examination of the placenta revealed membrane bound electron lucent lysosomes, which led to enzymatic and molecular diagnosis [73].

Failure to synthesize immunoprecipitable protective protein was found in fibroblasts of a patient with the early infantile disease [16]. There was no protective protein mRNA. In contrast, in the late infantile disease there was a larger quantity of the 54 kDa precursor protein and a trace amount of the mature 32 kDa protein. In a patient with the juvenile/adult disease [4] a small amount of normal-sized precursor was found [72], while others in this group made precursors of various molecular sizes from 45 to 63 kDa [74]. The gene was mapped to chromosome 20 by somatic cell hybridization [60] and *in situ* hybridization [18]. There are 15 exons over 7 kb of genomic DNA [75].

By 2013, only 23 mutations had been identified [76]. Japanese patients with adult mild clinical disease were found to have a deletion of exon 7 [29, 77]. This resulted from a substitution at the donor splice site of intron 7, which causes aberrant splicing of the precursor mRNA [22]. Patients with the genotype have had relatively more severe disease with juvenile onset. The exon 7 deletion was in compound with point mutations changing glycine 49 to arginine, tryptophan 69 to arginine, and tyrosine 395 to cysteine [22, 29, 78]. Adult, milder phenotypic patients with the exon 7 deletion are generally homozygous. Two patients with the late infantile disease [5, 36] were found to have mutations for phenylalanine 412 to valine [21]. Expression in COS cells led to a precursor that was to some extent retained in the endoplasmic reticulum, which would be consistent with the finding of increased precursor and shortage of mature protein in this form [72]. A number of Caucasian patients with this form of the disease have been found to have a point mutation changing tyrosine 221 to asparagine [79]. Others had phenylalanine 112 to valine 22 [80]. A total of 23 mutations in the *CTSA* gene have been reported in the international data base (http://portal.biobase-international.com/cgi-bin/portal/login.cgi).

In the rare infantile disease, three novel nucleotide changes have been reported [76], two of them missense mutations, c.347A>G (p.His116Arg) and c.775T>C (p.Cys259Arg); the third a nucleotide [75]. Another led to a stop codon (c.1216C>T) that led to p.Gln406 stop [76]. Two Dutch patients with this form of the disease had novel mutations p.Gly57Ser and a C insertion at 899 that led to a frameshift and premature termination [80]. Another patient with fetal ascities and hydrops had A>G (Q49G) at position 146 [82]. Early infantile patients have been found to have valine 104 to methionine, leucine 208 to proline, and glycine 411 to serine [81].

In a 24-year-old with the disease, a previously reported missense paternal mutation (67T>A) p.Y267N) was found along with a novel maternal mutation (c.886_887delTA) which caused a frameshift. This experience illustrated the utility of whole exome sequencing. Prior to this, the diagnosis had not been considered. Whole exome sequencing has been recommended after experience with a girl originally thought to have Williams syndrome and the p.Y267N variant [83].

In two Dutch early infantile patients' novel mutations p.Gly57Ser and insertion C at 899 led to a frameshift and premature termination [81]. This insertion was in homozygosity in the second patient. Homozygosity for Q49R led to the severe neonatally fatal phenotype [82].

In the *PPGB* gene, a novel missense mutation, (p.G86V) was found in Portuguese patients [24], as well as a previously reported p.V104M, and two novel deletions (c.230delC and c.991-992delT) coding for non-functional proteins. A missense mutation was also found at the cathepsin A active site.

The natures of the enzymatic defects in β-galactosidase and in neuraminidase lead to the accumulation in tissues of these patients of GM_1 ganglioside [43] and other gangliosides, including GM_3, as well as sialylated storage compounds as in sialidosis [84]. A variety of sialyloligosaccharides is excreted in the urine. Their detection by thin layer chromatography may be useful in leading to the diagnosis [26, 58, 85]. These results are followed up with enzyme assays to establish the diagnosis and, most often, the diagnosis is now made

by direct assay of the activities of β-galactosidase and neuraminidase.

Clinical diagnosis has often been made by thin layer chromatography for oligosaccharides [86]. The disease has been classified as an oligosaccharidosis. A Liquid Chromatography-Tandem Mass Spectrometry (LC MS/MS) method has been developed [85] for the unique pattern found in each of the oligosaccharidoses. Capillary high-performance anion-exchange chromatography with MS has also been employed in galactosialidosis [86] to detect free oligosaccharides. O-sulfated oligosaccharides were detected and glycan products of glycosphingolipids including aldohexonic products of oligosaccharides and reducing end hexose, suggesting alternative rates of catabolism.

Oligosaccharidoses result from a deficiency in enzymes responsible for the catabolism of protein-bound oligosaccharides and are typified by the accumulation of corresponding sugars in the urine. An effective screening and diagnostic tool for these disorders is by a mixed mode LC MS/MS assay which potentially mitigates many of the problems associated with thin layer chromatography. Each oligosaccharidoses produces a unique selected reaction monitoring fingerprint.

TREATMENT

Effective specific therapy has not been devised. The availability of animal models should permit studies on gene transfer as an approach to therapy. Bone marrow transplantation has been successful in PPCA (2/2) mice [87]. Chemical Chaperone therapy with N-Octyl-4-epi-β-valienamine (NOEV) was found [89] to stabilize and enhance β-galactosidase activity in cultured fibroblasts derivred from 4 patients.

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98

Metachromatic leukodystrophy

MAJOR PHENOTYPIC EXPRESSION

Delay or deterioration in walking, progressive neurodegenerative disease, optic atrophy, and grayish discoloration of the retina, symmetrical decrease in the density of cerebral white matter, elevated cerebrospinal fluid (CSF) protein, increased excretion of urinary sulfatide, and deficient activity of arylsulfatase A.

INTRODUCTION

Metachromatic staining of the brain in neurodegenerative disease was reported as early as 1910 by Perusini and by Alzheimer [1, 2] in studies of adults. The classic late infantile form of metachromatic leukodystrophy (MLD) was first reported by Greenfield [3] in 1933. In 1925, Scholz [4] published a detailed clinical pathologic study of juvenile or childhood-onset leukodystrophy, and 34 years later, Peiffer [5] demonstrated that the neural tissues of Scholz's frozen sections stained metachromatically. The metachromasia results from the accumulation of sulfatides, and this was discovered independently in 1958 by Jatzkewitz [6] and Austin [7]. It was Austin and his colleagues [8] who found the defective activity of arylsulfatase A (ASA). Mehl and Jatzkewitz [9] demonstrated defective activity against cerebroside sulfate, the material that accumulates in MLD (Figure 98.1).

ASA is the enzyme responsible for desulfation of the lipid component myelin. In MLD, there is intralysosomal storage of glycosphingolipid sulfatide, which leads to progressive neurologic disease [10].

The sulfatase enzyme is heat labile. A heat stable factor that increases activity several-fold is known as saposin B and, in rare instances, MLD results from defective activity of this protein, and arylsulfatase activity is normal [11, 12]. Deficiency of the heat stable factor causes deficiency of the enzymatic hydrolysis of sulfatide [13, 14]. Other variants with clinical MLD have signs of mucopolysaccharidosis and have been found to have multiple sulfatase deficiency (Chapter 99).

The gene for ASA has been localized distal to band q13 on chromosome 22 [15], and it has been cloned and sequenced [16]. A number and variety of mutations have been elucidated. In general, patients with late-infantile MLD have null mutations leading to absence of enzyme activity and immunoreactive enzyme either in patient cells or when the gene is introduced into animal cell-line expression systems [17]. Mutations which express small amounts of cross-reacting material (CRM) and active enzyme are found on at least one allele in juvenile onset and adult disease.

The diagnosis of MLD is complicated by the fact that there is a benign pseudodeficiency allele for ASA, which

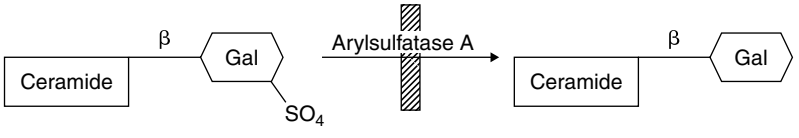

Figure 98.1 The arylsulfatase A (ASA) reaction, site of the defect in metachromatic leukodystrophy. CER, ceramide; GAL, galactose; SO_4, sulfate. A number of other sulfatides, such as lactosylceramide sulfate, as well as the galactosylceramide sulfate shown, are natural substrates for the enzyme.

in homozygotes leads to low ASA activity and no clinical abnormalities [18, 19]. Thus, not only can patients with MLD have normal activity of ASA (when the deficiency is in saposin B), but patients with little or no activity of ASA may not have MLD (when they have the pseudodeficiency allele in homozygosity).

CLINICAL ABNORMALITIES

MLD has been divided into three or five subgroups on the basis of the age of onset and rapidity of neurologic degeneration. In one classification, the proportions were the late-infantile (50–60 percent of patients); juvenile (20–30 percent); and adult (15–20 percent) [20]. As in the case of many genetic diseases, the advent of molecular biology may make all of these classifications obsolete, but it currently remains useful to distinguish at least the infantile and adult phenotypes. The classic late infantile disease (Figures 98.2 and 98.3) begins before 30 months of life and is progressive to death in one to seven years. MLD accounts for approximately 8.2 percent of the leukodystrophies of children [21]. The first manifestations are loss of acquired motor skills, especially walking, which becomes unsteady. Examination at this time reveals hypotonia, and often a pronounced genu recurvatum. Deep tendon reflexes are diminished or even absent, indicative of neuropathy. In some patients, walking is delayed, and some never learn to walk [22, 23], but most learn to walk unassisted and to speak short sentences, and then these skills deteriorate. Intercurrent infection may be followed by ataxia and weakness, which may disappear, but reappear later. The initial presentation with hypotonia and reflex changes may suggest a myopathy or peripheral neuropathy [24–26]. There may be intermittent severe pains in the legs [25]. Magnetic resonance imaging (MRI) [27] revealed diffuse enhancement cranial nerve and cauda equina nerve roots in a patient with infantile MLD [28]. Hagberg [25] has viewed

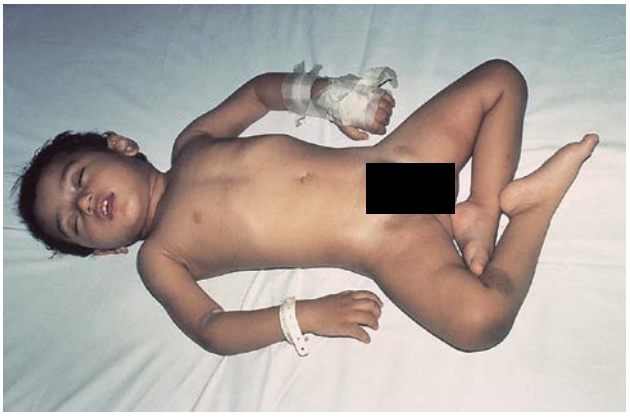

Figure 98.2 SAS: A 24-month-old infant with MLD was markedly hypotonic and assumed a frog-leg position. He had lost milestones achieved. He had markedly diminished deep tendon reflexes. ASA activity was <0.1 nmol/mg per hour.

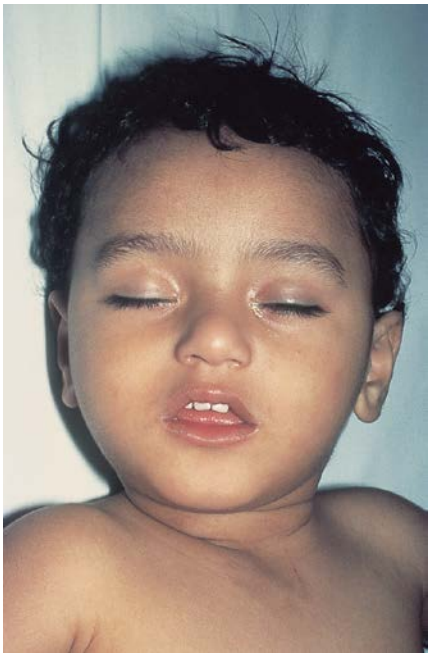

Figure 98.3 SAS: The closed eyelids symbolize loss of contact with surroundings. Cerebrospinal fluid (CSF) protein was 80 mg/dL. Nerve conduction velocity was diminished.

the progression of the disease in four stages, the initial picture representing stage I. In stage II, the patient is no longer able to stand, but can sit. There is ataxia and truncal titubation. Speech deteriorates and is dysarthric or aphasic, and mental function regresses. Muscle tone is increased in the legs and deep tendon reflexes are exaggerated. Ocular nystagmus develops, and ophthalmoscopy reveals optic atrophy and a grayish discoloration of the retina and macula, sometimes with a central red spot reminiscent of Tay-Sachs disease [26] (Chapter 88). In stage III, the patient develops spastic quadriplegia and is confined to bed. There may be decerebrate or decorticate rigidity or dystonic movements. Seizures develop in about one third of patients [25]. Pharyngeal muscle coordination is lost, and there is difficulty with feeding and with the airway. Mental deterioration continues, and speech is lost. The child may continue to respond to parents and smile. In stage IV, contact is lost. The patient is blind and cannot swallow. Tube feeding is required. Death results usually from pneumonia. In 29 Brazilian patients with a spectrum of phenotypes, the first initial manifestation of disease was disturbance of gait or other motor abnormality (72.7%) in the late infantile form and behavioral or cognitive abnormalities (50%) in the juvenile form [29].

Juvenile MLD has been the designation of patients where initial presentation was between four and 16 years of age, often with a decrease in school performance in the first or second grade, sometimes with unusual behavior [30]. The patient may appear confused or to be daydreaming. Some have had dementia, psychosis, or emotional illness. Younger patients may present with clumsiness of gait, as

in the infantile patients [28]. Muscular rigidity, postural abnormalities, and ataxia may occur. Within one year of onset, the patient is unable to walk. Urinary incontinence may occur early. Progression is to stages III and IV, as in the late infantile disease. It is clear that patients within this group may have phenotypes overlapping those of younger and older patients; the distinction may be artificial. In fact, instances have been described of siblings in the same family with juvenile and adult disease [31–35]. Some unusual visceral presentations have been with acute cholecystitis [31], chronic hemorrhagic pancreatitis [36], abdominal mass [37], or gastrointestinal bleeding [38]. There may be polyps in the intestine and in the gallbladder [39].

Adult MLD refers to patients presenting after puberty [40]. Onset may be as young as 15 years of age [41] or as late as 62 years. Survival may be for five or ten years or longer. Symptomatology is largely psychiatric. The recognition of these patients is a strong argument for neuroimaging studies in psychiatric patients [42]. Dementia may be manifest in loss of memory or decrease in intellectual ability. Psychotic changes may be those of schizophrenia. There may be emotional lability, anxiety, or apathy. Visual–spatial discrimination may be impaired. Auditory hallucinations and delusions were reported in 18 and 27 percent of patients, and psychosis in 53 percent [43]. Depression and chronic alcoholism have been observed.

Motor disturbances may develop with clumsiness of gait and dysarthria. Muscle tone increases and deep tendon reflexes are brisk. Some develop ataxia and some have Parkinson-like features. In some patients, the initial manifestations are those of peripheral neuropathy [44, 45]. Dystonic movements may develop. Degeneration progresses to spastic tetraparesis, bulbar involvement, and decorticate posturing. Optic atrophy and nystagmus are found. There may be seizures. Ultimately, the patient is blind, mute, and unresponsive. Gross Motor Function Classification (GMFC-MLD) has been standardized as a tool for the assessment of gross motor function in MLD, description of the natural course of the disease, and evaluation of therapy [46].

Patients with MLD as a result of deficiency of the cerebroside sulfatase activator, saposin B, have generally presented as juvenile MLD [11, 12, 47]. In one patient, onset was at 48 years with intellectual deterioration, apathy, and withdrawal [48]. Each was recognized initially on the basis of an MLD phenotype with normal activity of ASA [13].

The clinical laboratory evaluation of patients with established MLD is notable for elevation of the concentration of protein in the CSF. The level may be normal early in infantile disease, but it rises progressively to levels of 100 mg/dL or higher. This is also true for the younger-onset juvenile patients; while later-onset juvenile and adult-onset patients usually have normal levels of protein, though there have been a few with elevated concentrations [43]. CSF concentrations of cytokines have been elevated in this disease [49]. Significantly elevations of IL-1Ra, IL-8, VEGF and others were found.

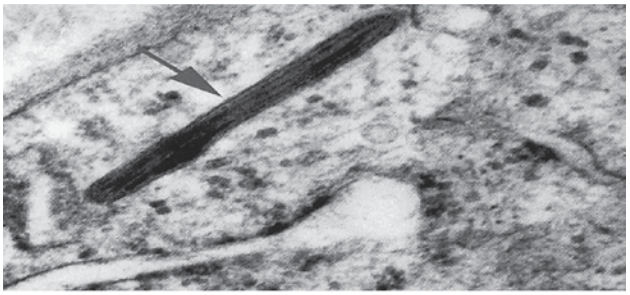

Figure 98.4 Electron microscopy of biopsied sural nerve indicating the dense inclusion body. These inclusions have been called zebra bodies because of the stripes.

The electroencephalograph (EEG) may be abnormal, especially in those with seizures [48–51]. There may be diffuse slowing or spike discharges, often focal. Noise may induce a marked startle response. The EEG tends to be normal in the adult-onset patients [51].

Motor nerve conduction is slowed [28]. These abnormalities have been demonstrated in presymptomatic patients, indicating the presence of peripheral neuropathy well before the onset of symptoms. Delay may be evident in afferent nerves before that of efferent nerves [52–55]. There may also be abnormalities in brainstem auditory evoked responses (BAER), visual evoked responses, or somatosensory responses. Abnormalities in BAER may be evident when peripheral nerve conduction is unimpaired [56]. Biopsy of peripheral nerve reveals the characteristic inclusion bodies (Figure 98.4). Neuroimaging by computed tomography (CT) or magnetic resonance (MR) is consistent with loss of myelin and increase in water. Low densities on CT and hyperintense T2 images on MRI are visible in periventricular white matter indicative of leukodystrophy (Figure 98.5) [57, 58]. Later, there is evident atrophy. Proton magnetic resonance spectroscopy (MRS) reveals reduction in N-acetylaspartate and increase of myoinositol, a glial marker [59]. N-acetylaspartate (NAA) is a marker for loss of neurons and axons. It can be measured *in vivo* by proton MRS. In MLD, the NAA signal was reported to decrease during progression of the disease [60]. Screening test for lysosomal storage diseases was successfully carried out using vacuolated peripheral lymphocytes. Lysosomal protein profiling has been employed [61] via high throughput assay of dried blood spots. Positive identification was obtained in 99 percent of patients, including MLD.

GENETICS AND PATHOGENESIS

MLD is inherited in an autosomal recessive fashion. Multiple affected siblings of both sexes and normal parents have often been observed, and consanguinity was noted early [62]. The incidence of the late infantile form has been estimated at one in 40,000 in Sweden [63]; the juvenile form is about four times less common. An unusually high

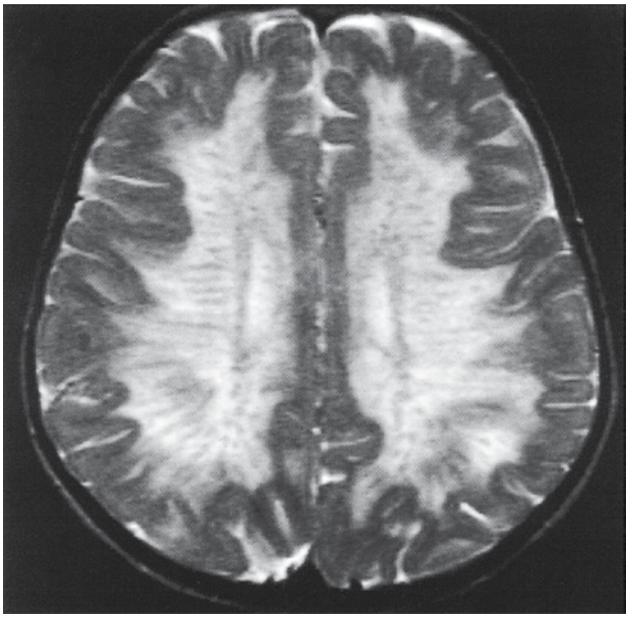

Figure 98.5 MRI of the brain of a five-year-old female with MLD. There was diffuse high T_2 signal throughout the white matter in a sunray appearance. She had regressed developmentally and had diminished deep tendon reflexes.

incidence of late infantile MLD of one in 75 live births was reported in an isolate of Habbanite Jews [64].

The molecular defect is in ASA [8], which acts in tissues as the cerebroside sulfatase (Figure 98.1). This acidic glycoprotein enzyme is synthesized as a 62-kDa precursor protein, and then translocated via a mannose-6-phosphate receptor to the lysosome as 57–62 kDa forms [65]. The deficiency has been demonstrated in many different tissues, including brain, cultured fibroblasts, leukocytes, and urine [66–70]. The enzyme activity is usually measured against artificial substrate, such as p-nitrocatechol sulfate; the assay usually reveals some residual activity. Immunochemical studies have revealed CRM-positive and CRM-negative examples of late infantile MLD [71, 72]. There is evidence of rapid degradation of synthesized enzyme in juvenile- and adult-onset MLD. Studies of the degradation of cerebroside sulfate in intact fibroblasts have yielded correlations between the effectiveness of the cells in catabolizing sulfatide and the age or sex and severity of the clinical phenotype [33, 73]. ASA activity is also defective, along with those of other sulfatases in multiple sulfatase deficiency (Chapter 99).

ASA activity is normal in patients with MLD that results from the activator saposin B [14], but cultured cells from these patients fail to degrade added sulfatide. Addition of purified activator protein corrects this defective behavior, and immunologic study reveals an absence of CRM against saposin B protein [47, 74, 75].

Pseudodeficiency of ASA was first identified through the testing of clinically normal relatives of patients with MLD [76–78]. These individuals have a pseudodeficiency gene (pd), which leads to ASA activity of 5–15 percent of normal [71]. They do not have sulfatiduria or storage of metachromatic material. The protein is kinetically normal, but smaller in size, and it lacks a glycosyl subunit.

The gene for ASA is on chromosome 22 at q13 [15, 78–80]. The saposin B gene is on chromosome 10 [81, 82]. The ASA gene consists of eight exons in a small 3.2-kb coding area [83]; the mRNA is 2.1 kb. A large number of mutations has been found for ASA [17, 84, 85]. A common polymorphism leads to an enzyme with perfectly normal activity and a threonine to serine change in exon 7 [17]. In late infantile MLD, the mutant alleles are sometimes referred to as I-type mutations [17]. More often, these are now referred to as null alleles, which code for no enzyme activity. Two splice-site mutations have been identified that lead to this phenotype. They include the G609A transition that destroys the splice donor site of exon 2 by changing the exon–intron boundary from AGgt to AGat [17]. This common mutation in Europeans has also been seen in Arabs. Another was a G2195A transition at the splice-recognition site between exon 7 and the next intron [86]. In addition, an 11-bp deletion in exon 8, which causes a frameshift, was also found in this phenotype [87]. Point mutations have also been found in this phenotype, including a glycine 99 to aspartic acid change in exon 2 [88], common in Japan, and a glycine 245 to arginine change in exon 4 [89]. Other common mutations among Europeans are P426L [17] and I79S [84]. Mutations such as the G-to-A transition in exon 2 which results in a change from glycine 99 to aspartic acid [90] and the proline 426 change to leucine have been referred to as type A mutations and in the homozygous situation lead to adult-onset MLD. They are now referred to as R alleles and they code for some residual activity. In 26 patients in Italy [91], the c.459+1G>A mutation was found in 19 of 52 alleles (36.5%). There were ten rare mutations and eight newly found. By 2016, 200 mutations in the ASA gene have been recognized [92], the majority single point mutations leading to missense. There has been a clustering of mutations around exon 2 and 5. The most common alleles were c.465+1G>A, c.1283C>T, and c.542T>G. In a report of 16 Saudi patients with MLD, seven patients had ASA-deficiency, and four had sphingolipid activator deficiency. The latter four were homozygous for a g.722G>C transversion which led to a p.C241S previously reported mutation [93] and four have sphingolipid. Compound heterozygosity for the mutation (p.G99D and p.T409I0) was found in a Japanese female with behavioral abnormalities [94]. Nine pathogenic mutations were found in 13 Indian patients (65 percent), five of them novel. The most common mutation was c.459+16>A [95]. Compounds of A- and I-type mutations have been found in juvenile-onset patients [17]. In a Korean boy who could not walk at 12 months and died at nine years, a novel splicing mutation (c.1101+1G>T) in intron 6 was found on one allele, as well as a missense mutation in exon 2 (c.296G>A; p.Gly99Asp) [96].

The pseudodeficiency enzyme is 2–4 kDa smaller than normal enzyme. This is a result of a point mutation

(asparagine to serine) at the C-terminal glycosylation site leading to loss of an oligosaccharide side chain [97]. The Pro426Leu mutation, second most common in Europeans, codes for an enzyme that is synthesized normally and targeted normally to the lysosomes, but there it is promptly degraded [98]. Two R alleles are usually found in the adult-onset disease [17, 82, 99]. Patients with I179S on one allele usually begin with psychiatric manifestations, while those carrying P426L usually begin with a neurologic picture [100].

The molecular biology of the saposin B activator has also been clarified. A prosaposin precursor gene directs the synthesis of a precursor protein from which the individual saposins are derived [101]. Mutations have been identified including a C-to-T transition leading to a substitution of isoleucine for threonine that eliminates a glycosylation site with a neighboring asparagine [102] in the original family of Shapiro and colleagues [11].

Heterozygote detection has been accomplished by assay of ASA activity in leukocytes and fibroblasts [103]. Overlap with the normal range made the designation of noncarrier less reliable. The pseudodeficiency allele is common [14] and this may even suggest that a relative is affected. Testing with sulfatide-loaded fibroblasts may be required for resolution [101]; molecular detection of the pseudodeficiency pd allele will also resolve this. The pd allele causes two A-to-G mutations, changing arginine 352 to serine, with loss of a glycosylation site, and the change of a polyadenylation signal. In families in which the mutation is known, its detection is best used for heterozygote elucidation. Searching for the most common splice-site mutation in late infantile MLD may be particularly useful.

It may also be employed for prenatal diagnosis. So far, enzyme assay has been employed for this purpose [70, 104, 105]. Prenatal diagnosis has been accomplished with cultured amniocytes and chorionic villus material. Here, too, the pseudodeficiency allele is a problem that must be recognized and dealt with. Sulfatide loading is usually helpful.

The consequence of cerebroside sulfatase deficiency is the accumulation of sulfatides in tissues, notably the cerebral white matter [6, 7, 106]. In addition to sulfatide, lipid lysosulfatide, which has been found in MLD, is cytotoxic in cell culture. High performance liquid chromatography revealed accumulation of lysosulfatide in the brain of arylsulfatase null mutant mice [107].

The accumulated sulfatide leads to decreased content of cerebrosides and the other lipid components of myelin. Sulfatide is also found in increased quantity in the urine [108]. The amounts may be 100–200 times the normal level [109]. This property has sometimes been employed to identify patients for testing for saposin B deficiency in those with clinical MLD and normal ASA activity. Patients with pseudodeficiency do not have sulfatiduria [110].

The demyelination that characterizes the disease is doubtless a consequence of the accumulated sulfatide. Neuropathologic changes have even been seen in fetuses at the fifth month of presentation. In particular, the oligodendroglia and Schwann cells appear to be targeted.

TREATMENT

Treatment of patients with MLD has been largely supportive, including conventional treatment of seizures, prevention of contractures with muscle relaxants, physical therapy, and family support [111]. Vigabatrin may be useful in reducing spasticity [112].

Bone marrow transplantation has been employed in a number of patients [113–117]. Normal levels of circulating ASA have been achieved, and the clinical course has seemed to slow, particularly as compared with that of an affected sibling [114, 115]. It appears most useful in presymptomatic or early symptomatic patients. It may even accelerate progression in rapidly deteriorating patients [7, 111]. In ten families in which presymptomatic diagnosis was made because a previous sibling had had disease, there was successful engraftment in each [116]. Best results were in juvenile and adolescent forms. Adults with psychiatric disease may benefit from transplantation [116]. Transplantation is currently not recommended for symptomatic early onset forms of the disease [117].

Transplantation of bone marrow cells expressing the homeobox B4 in mice with MLD led to immunohistologic evidence of enzyme in microglia and improvement in ability to walk [118]. Hematopoietic stem-cell transplantation was without clinical improvement in MLD despite chromosomal and normal leukocyte arylsulfatase activity [118]. The results of allostem cell therapy in five patients with adult-onset MLD were reported to be poor [119]. The outcome of unrelated umbilical cord blood transplantation in three siblings with juvenile MLD was reported to be an inhibition of progression and stabilization of disease [120].

Genetically modified (to overexpress ASA), autologous, hematopoietic stem cell therapy has been recommended [121].

Enzyme replacement therapy (ERT), which has been successful in many lysosomal storage diseases notably Gaucher disease (Chapter 90), has not been successful in disorders such as MLD with prominent cerebral manifestations because of the efficiency of the blood–brain barrier. Infusion of recombinant human arylsulfatase A (rhASA) by an implanted miniature pump into the cerebral ventricular fluid of knockout mouse models of MLD indicated penetration of brain parenchyma and targeting to lysosomes. Histologic examination indicated reversal of lysosomal storage, and correction of ataxic gait [10]. In a mouse model intravenous injection of rhASA at 20 mg/kg weekly for four weeks reduced storage in the brain [122]. In an *in vitro* model system, porcine brain capillary endothelial cells were developed as an approach to increasing blood to brain transfer of sulfatase and improve therapeutic efficacy of enzyme replacement therapy [123].

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99

Multiple sulfatase deficiency

MAJOR PHENOTYPIC EXPRESSION

Facial and somatic features and dysostosis multiplex of a mucopolysaccharidosis; ichthyosis; neurologic features of a late infantile metachromatic leukodystrophy; mucopolysaccharidoria; defective activity of arylsulfatase A, B, and C, steroid sulfatase and the mucopolysaccharide sulfatases, including iduronate sulfatase, heparan-N-sulfatase, N-acetylgalactosamine-6-sulfatase, and N-acetylglucosamine-6-sulfatase; and defective post-translational enzymatic change of sulfatase cysteine-69 to aminopropionic acid.

INTRODUCTION

Multiple sulfatase deficiency (MSD) was reported in 1965 by Austin, Armstrong and Shearer [1] as a metachromatic leukodystrophy (MLD) in which there were also features of mucopolysaccharidosis. The disease has been referred to as Austin disease. Deficient activity of a number of sulfatases led to the designation of MSD [2]. At least seven enzymes are now known to be deficient [2–5]. The fundamental defect [6– 7] represents a novel mechanism of disease in which the mutation is in an enzyme responsible for post-translational change of a cysteine moiety of each of the sulfatases, a change that normally conveys activation of the enzyme (Figure 99.1). This cysteine is conserved in each of the sulfatases (Table 99.1). It is converted to 2-amino-3-oxopropionic acid (formylglycine) (Figure 99.1) [8].

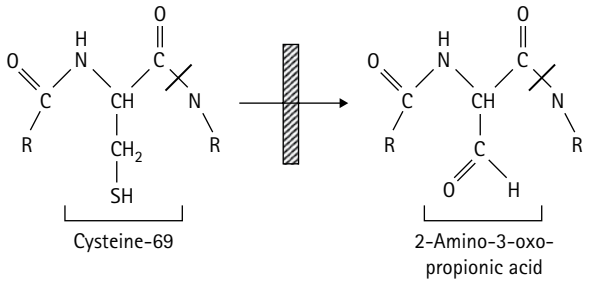

Figure 99.1 Mechanism of activation of sulfatase enzymes by conversion of a highly conserved cysteine at position 69 of sulfatases to 2-amino-3-oxopropionic acid.

Table 99.1 Homology of sulfatase sequences

Arylsulfatase A	L	C	T	P	S	R
Arylsulfatase B	L	C	T	P	S	R
Steroid sulfatase	L	C	T	P	S	R
N-Acetylglucosamine-6-sulfatase	L	C	C	P	S	R
Iduronate sulfatase	V	C	A	P	S	R
N-Acetylgalactosamine-6-sulfatase	L	C	S	P	S	R
Sea urchin arysulfatase	V	C	T	P	S	R

This post-translational change affects the entire sulfatase family, at least seven members of which are lysosomal enzymes that are specifically involved in the degradation of sulfated glycosaminoglycans, sulfolipids, or other sulfated molecules. It leads to combined deficiencies of all of the sulfatases.

The enzyme, formylglycine-generating enzyme (FGE), catalyzes the oxidization of the cysteine residue at position 64 to 2-amino-3-oxopropionic acid. This is the amino acid which enables the sulfate ester hydrolysis. It is located in the ER [8].

The gene has been identified, and missense mutations have been discovered which lead to variable loss of function [9]. The gene sulfatase modifying factor 1 (*SUMF1*) encodes

the enzyme, which normally activates sulfatases by modifying this key cysteine residue within the catalytic domains of each sulfatase. In patients with MSD, the mutated enzyme is retained in the endoplasmic reticulum (ER). This is the site of its enzymatic action on nascent sulfatases. *SUMF1* interacts with protein disulfide isomerase (PDI) and ERp44, two thioredoxin family members of the early secretory pathway, and with ERGIC-53, a lectin that shuttles between the ER and the Golgi. Functional assays reveal that these interactions are crucial for controlling *SUMF1* traffic and function [10].

Deficiency of arylsulfatase A would be consistent with clinical features of MLD. The deficiency of steroid sulfatase is responsible for the skin lesions of X-linked ichthyosis. Among the enzymes of mucopolysaccharide metabolism: (1) deficiency of iduronate sulfatase provides manifestations of Hunter syndrome; (2) deficiency of heparan sulfatase yields the impaired mental development and cerebral features of Sanfilippo A disease; (3) deficiency of N-acetylglucosamine-6-sulfatase, those of Sanfilippo B disease; (4) deficiency of N-acetylgalactosamine-6-sulfatase gives rise to features of Morquio disease; and (5) deficiency of N-acetylgalactosamine-4-sulfatase, also known as arylsulfatase B, causes features of Maroteaux-Lamy disease, including corneal clouding. Obviously, different degrees of deficiency or amounts of residual enzyme activity would be expected to lead to quite different phenotypes. A number of different clinical phenotypes have been delineated [4], including the classic late infantile form, a neonatal form, a juvenile form, and a Saudi variant.

CLINICAL ABNORMALITIES

The clinical phenotype of MSD represents a summation of the various enzymatic defects [4, 5, 7, 11] a combination of the clinical features found in diseases resulting from deficiency of the individual sulfatases: mucopolysaccharidoses II, IIIA, IIID, IVA, and VI, MLD, X-linked ichthyosis, and the X-linked recessive form of chondrodysplasia punctata [12]. The phenotypic outcome in MSD depends on the degree of residual FGE enzyme activity and on protein stability.

In its classic form, the disease presents in late infancy with the symptoms of the progressive degeneration of myelin of MLD [11], or in the neonatal form with a picture of a severe mucopolysaccharidosis [12]. A milder juvenile form with onset at about five years of age has been reported [6].

The more commonly described presentation is that of an MLD [5, 7, 11] with mild features of mucopolysaccharidosis [13, 14]. Early development may be normal, and patients may walk and speak at normal times [4], but some may be developmentally delayed early on. During the second or third year, milestones attained are slowly lost. Increased deep tendon reflexes and ankle clonus may be followed by spastic quadriparesis. There may be seizures. Neurodegeneration is progressive to blindness and loss of hearing [11, 15], and deafness may be severe. Microcephaly develops [9, 16, 17]. Swallowing becomes difficult, and tube feeding is required. Death may occur at 10–18 years, but there has been survival into the third decade.

Mucopolysaccharidosis-like features maybe evident very early in life [12, 18], and may well be the first evidence of disease. The diagnosis should be considered in young patients with signs of mucopolysaccharidosis. The facial features are coarse, and there is hirsutism (Figures 99.2–99.5). There may be stertorous breathing, nasal discharge, or hernias. Hepatosplenomegaly may be prominent (Figure 99.6). Joints become stiff and there may be contractures. The claw hand may be identical to that of Hurler disease (Figures 99.7 and 99.8). Virtually all patients have roentgenographic evidence of dysostosis multiplex (Chapters 76 and 78). The initial diagnosis may be of Hunter or Sanfilippo disease [5, 18]. Cardiac complications have been observed [12]. A few patients do not appear to have recognizable clinical features of mucopolysaccharidosis. Ophthalmologic findings have included optic atrophy, retinal degeneration, and nystagmus [5, 10, 19]. The cornea is usually clear [5, 9, 18]. Two patients have had a cherry red macula [13, 20]. The classic presentation is with ichthyosis (Figure 99.4) [5, 16, 17].

Another neonatal phenotype has been distinguished [4] in which there is presentation at birth with prominent features of mucopolysaccharidosis, severe encephalopathy, and early demise [12, 15, 21]. Hepatosplenomegaly is pronounced. These patients have also had ichthyosis by two to three years of age [12, 21]. The neck is short, and there is hypoplasia of the vertebral bodies and epiphyseal

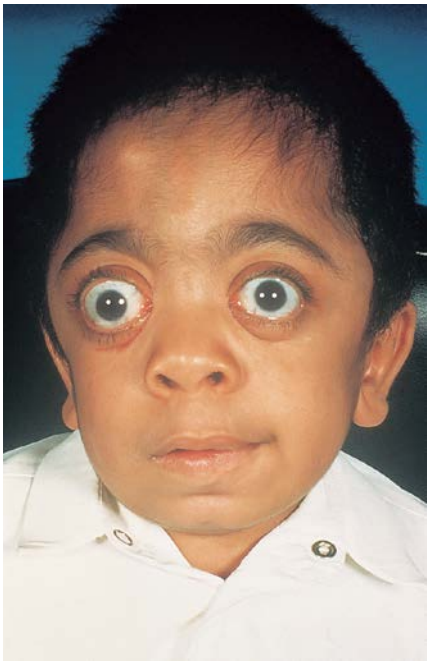

Figure 99.2 Multiple sulfatase deficiency (MSD). A patient with MSD whose eyes were strikingly proptotic. He also had atlantoaxial dislocation. Sulfatase activities were very low.

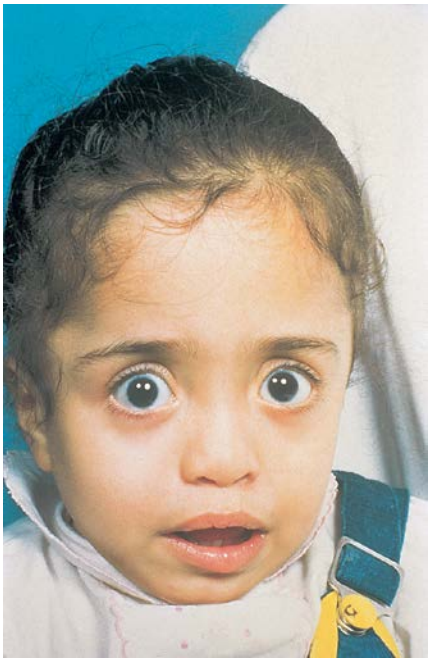

Figure 99.3 IQ: This girl with MSD also had strikingly prominent eyes.

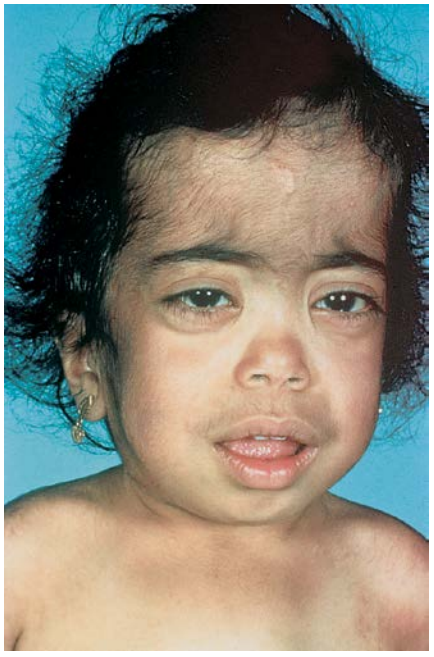

Figure 99.5 HZ: A girl with MSD. Hirsutism was very pronounced and the facial features coarse.

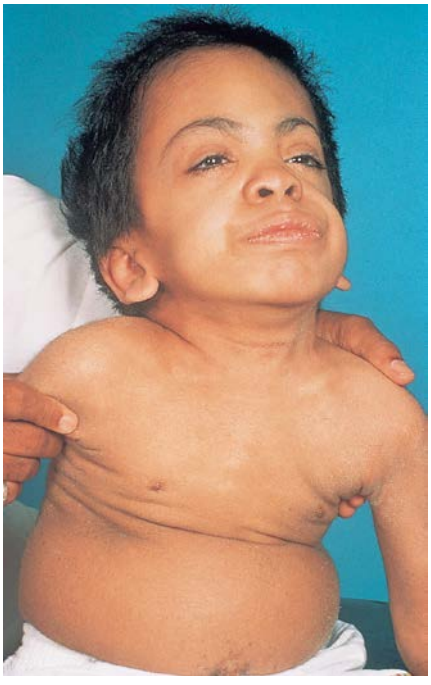

Figure 99.4 MM: A boy with MSD. Facial features were coarse, the nasal bridge depressed, and the nasal tip tilted, highlighting the abundant nasal subcutaneous tissue. The skin of the legs, as well as the torso illustrated, was ichthyotic.

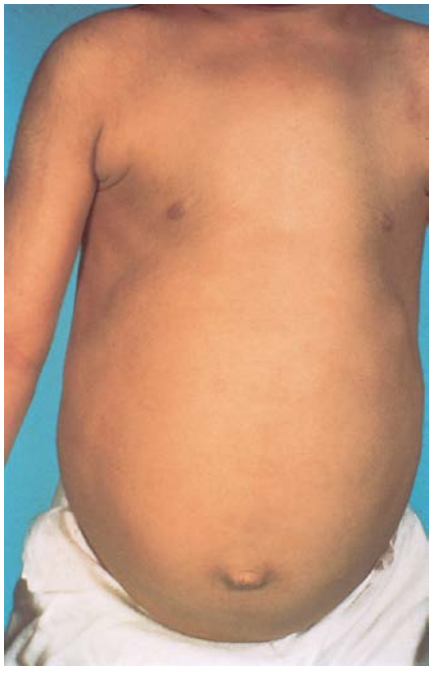

Figure 99.6 HZ: The abdomen was protruberant as a consequence of hepatosplenomegaly.

dysplasia. Consistent with the severity of the phenotype, enzyme activities of all the sulfatases tested were very severely depressed [12, 21].

Abnormal teeth were reported [22] in a girl who died at 13, and have been proposed as an early (potentially at birth) diagnostic tool. The teeth have sharp cusps, thin hypomineralized or missing enamel and exposed discolored dentin.

A Saudi variant has been distinguished [4] in which there was early infantile onset of severe dysostosis multiplex, appearing as Maroteaux-Lamy syndrome or Morquio disease. There was corneal clouding in six of eight patients. Facial

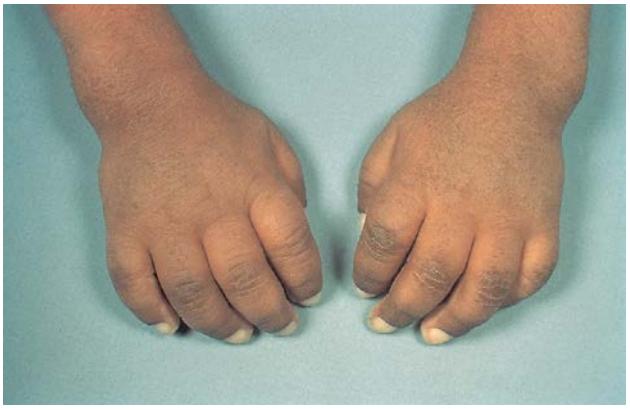

Figure 99.7 MM: The hands were just like those of a patient with Hurler disease.

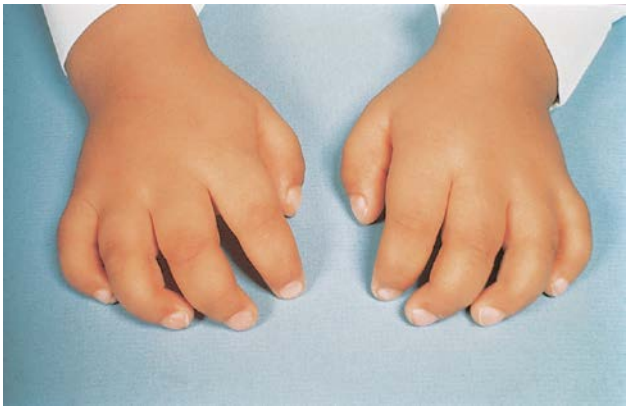

Figure 99.8 AA: Another patient with MSD and very striking hands. A brother also had MSD.

features were coarse. The orbit may appear shallow and the eyes proptotic (Figures 99.2, 99.3, and 99.15). Deafness was absent, but one patient had abnormal auditory evoked potentials on one side. Ichthyosis was absent, and in six of seven patients studied the activity of steroid sulfatase was normal [4]. On the other hand, we have since seen ichthyosis in another Saudi patient. Patients had mild to moderately impaired mental development, and one patient had a normal cognitive quotient despite motor impairment. There may be macrocephaly (Figures 99.10 and 99.11) and gingival hyperplasia (Figure 99.9). Most of these patients had retinal changes, but two had lenticular opacities, which have been seen, but rarely in the classic presentation [5]. Stature was short (Figures 99.12–99.15), often with a crouching, Morquio-like position because of contractures. Two patients had evidence of cervical cord compression with the development of sudden quadriparesis, followed in one by death.

A juvenile-onset form of MSD was reported by Tanaka *et al.* [23] in which there was onset at five years of a slowly progressive quadriplegia, retinitis, and blindness. There was ataxia and dysarthria. Hepatomegaly was only moderate. Stature was short and the skin was ichthyotic. Death was at 26 years.

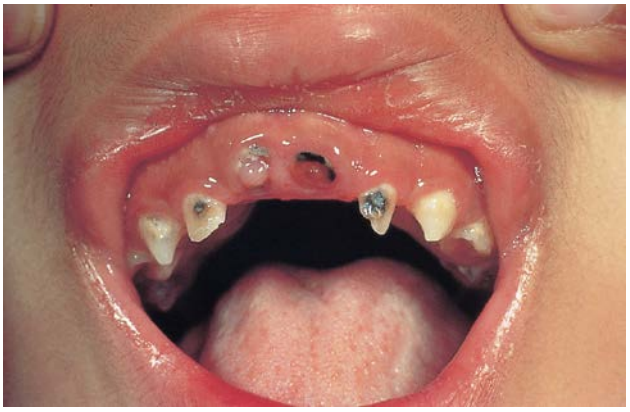

Figure 99.9 AA: The gingival hyperplasia was as striking as that seen in I-cell disease (Chapter 83). The teeth were carious.

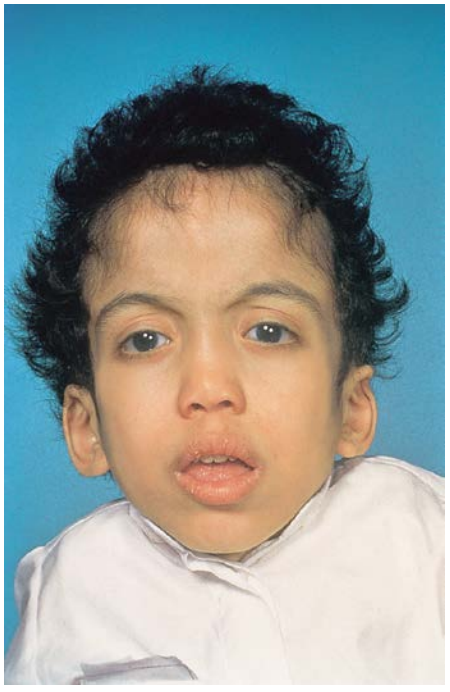

Figure 99.10 AR: A patient with MSD had an unusually shaped head and a prominent keel or frontal bridge.

Roentgenograms usually reveal some degree of dysostosis multiplex (Figures 99.17 and 99.18). In the Saudi patients, premature synostosis of one or more cranial sutures led to deformities, such as trigonocephaly, brachycephaly, or dolichocephaly [4]. Macrocephaly was seen, as well as the microcephaly more common in other forms of MSD. Some patients have had J-shaped sellas. Abnormalities of the odontoid have been observed [4]; C1 has been lower than normal. The posterior arch has been anterior, compressing the cord. There has been anterior subluxation of the atlas and hypoplasia of C2. A four-year-old girl with microcephaly had neurologic regression and spondylolisthesis, but no ichthyosis or coarse facies [24]. Nerve conduction may be slowed.

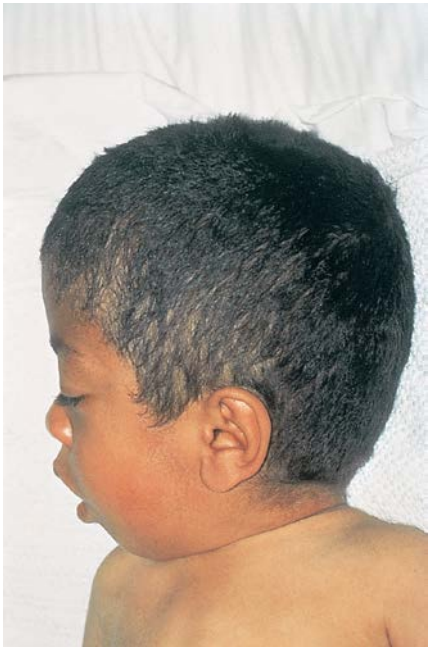

Figure 99.11 MM: A patient with MSD who was macrocephalic. Facial features were coarse, and he was hirsute.

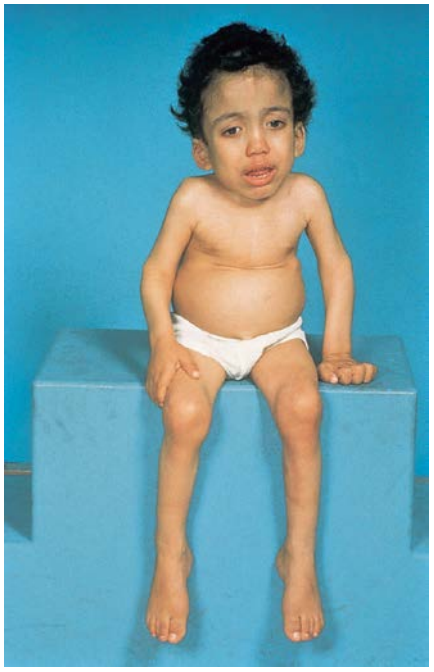

Figure 99.13 AQ: A patient who also had Morquio-like habitus.

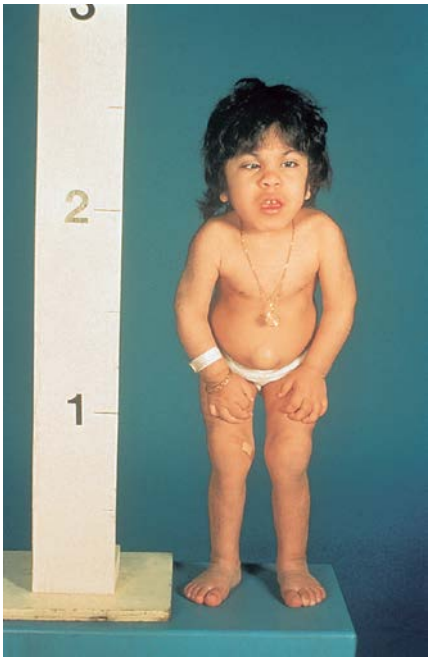

Figure 99.12 Stature was short and the neck particularly short. Flexion of the hips and knees, as well as the elbows contributed to the Morquio-like appearance.

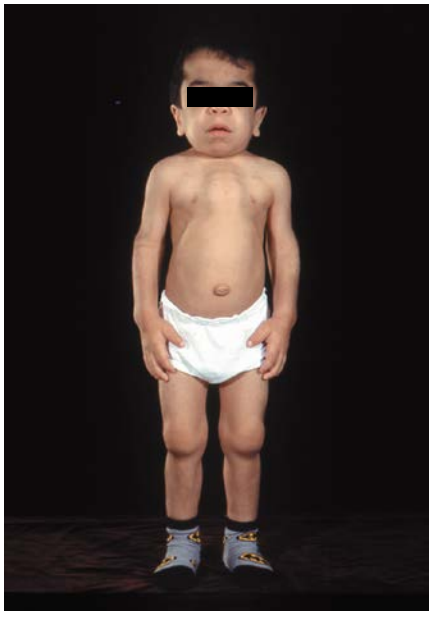

Figure 99.14 Seven-year-old boy with Morquio-like stature and coarse face, typical of Austin disease.

Neuroimaging of the brain by computed tomography (CT) or magnetic resonance imaging (MRI) (Figures 99.16–99.20) reveals a symmetric decrease in attenuation of white matter, with high T_2 signal throughout the white matter. White matter changes were seen in all the Saudi patients [4]. One had hydrocephalus and one an arachnoid cyst. MRI in a nine-month-old child with MSD demonstrated extensive diffuse symmetrical high signal in the deep white matter of both cerebral hemispheres, as well as of the subcortical white matter and the brainstem, while there was additional enlargement of sulci and subdural spaces and mild atrophy [25].

Laboratory findings in all these patients include mucopolysaccharidurie (dermatan sulfate and heparan sulfate). Alder-Reilly granules are found in leukocytes of the

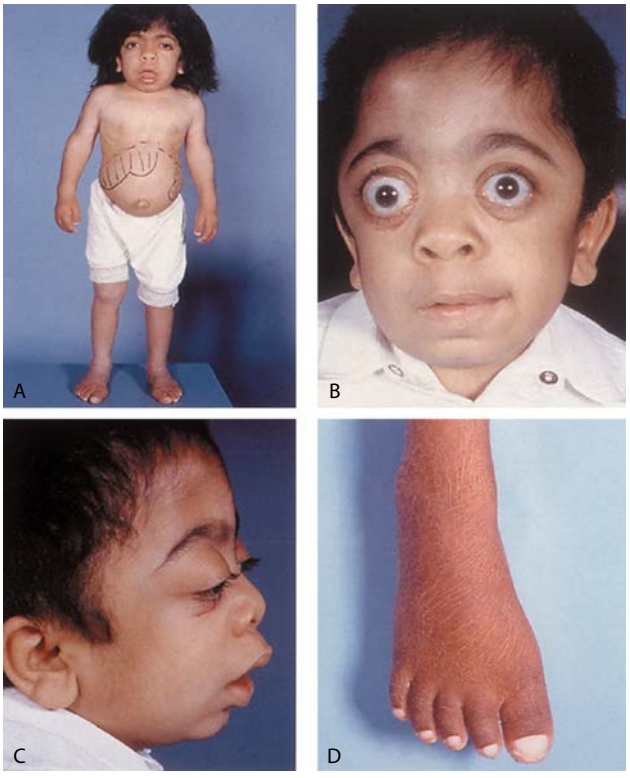

Figure 99.15 Phenotypic features of Austin disease. Typical stature (Morquio-like) (A). Typical face with coarse features (B and C), and ichthyosis (D). (B) is the same as Figure 99.2.

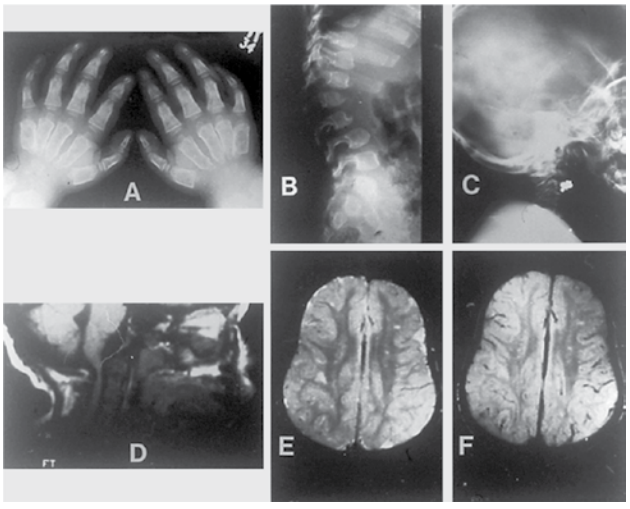

Figure 99.16 Radiological features of Austin disease, with typical hands (A), gibbus deformity (B), J-shaped sella (C), odontoid hypoplasia with C1–C2 posterior dislocation (D), and leukodystrophy (E and F).

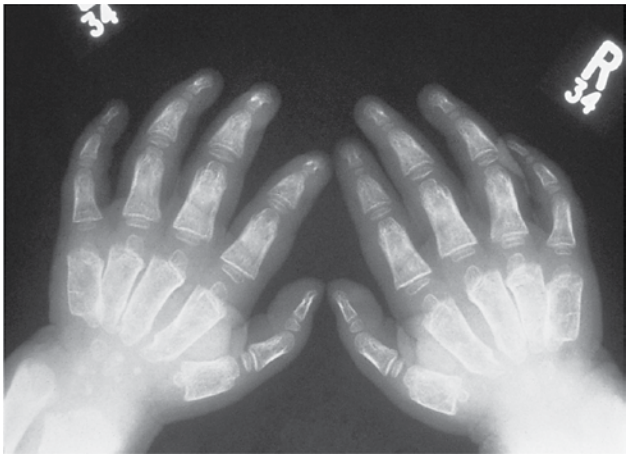

Figure 99.17 The hands of RI (Figure 99.12) illustrated the characteristic broad, short proximally tapering metacarpals and broad phalanges.

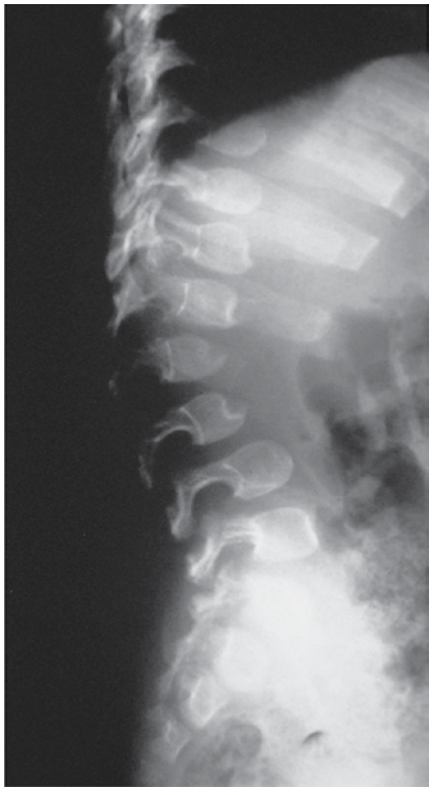

Figure 99.18 Roentgenogram of the spine of RI, illustrating advanced dysostosis multiplex. The ribs were broad and spatulate. The vertebrae were ovoid, and the lumbar vertebrae had anterior hooks.

bone marrow and peripheral blood. The cerebrospinal fluid (CSF) protein concentration may be elevated. Diagnosis is made by confirming the deficiency in the activity of a number of sulfatase enzymes [12].

Newborn screening for lysosomal storage diseases has been developed via quantification of immunoreactive lysosomal proteins [26]. A multiplex assay for 14 enzyme proteins studied 1415 dried blood spots and identified a patient with MSD on the basis of reduced amounts of many sulfatase proteins. In this study, sensitivity and specificity were good; there was one false negative, a patient with mucopolysaccharidosis II [27].

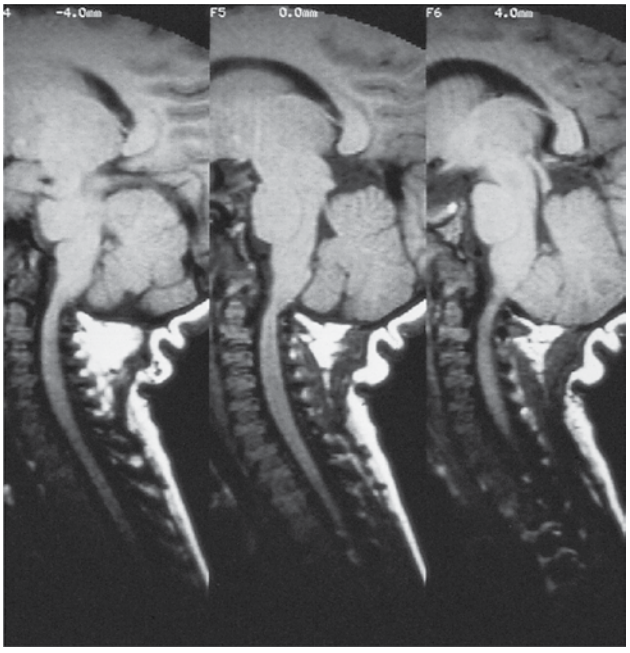

Figure 99.19 MRI of RF, a patient with MSD. A localized quite marked constriction of the anteroposterior diameter led to compression of the cord to about one-half normal size.

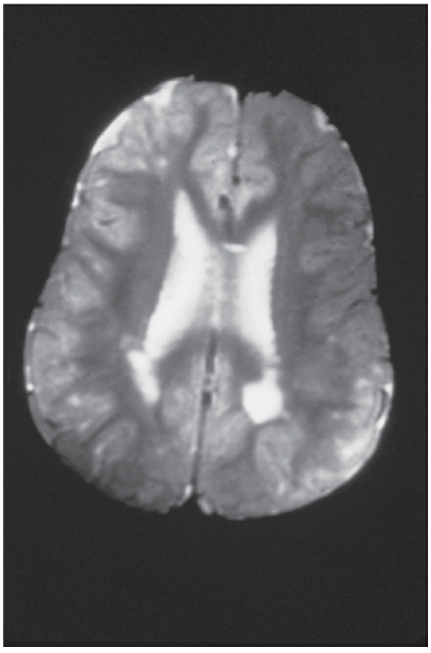

Figure 99.20 MRI of the brain of SR, a four-year-old patient with MSD. Moderately extensive high T_2 changes in the subcortical white matter were present bilaterally.

GENETICS AND PATHOGENESIS

Transmission is autosomal recessive. Both sexes have been equally represented, often with more than one affected patient in a sibship. Consanguinity has been documented. Prevalence in Australia has been reported as one in 1.4 million [28]. Less than 50 patients have been reported.

The locus has been shown by complementation studies in somatic cell hybrids to be different from that of MLD. Fusion of MLD and MSD fibroblasts led to correction of arylsulfatase A deficiency [29]. Hybridization of cells of the classic and neonatal forms of MSD was not complementary, and enzyme activity was not restored [30].

Defective activity of sulfatases can be shown in cultured fibroblasts [18, 19] and in tissues, such as kidney, brain, and liver [2, 12, 31]. There has been some correlation of the levels of residual enzyme activity and clinical phenotype [3, 29, 32]. In general, classic patients have had severe deficiency [3, 33, 34], while activity is absent or certainly less than 10 percent of control in the neonatal patients [21, 33]. In the Saudi patients, activities ranged from 3–10 percent of arylsulfatase A to 20–41 percent of control of the Sanfilippo A enzyme [4].

In the juvenile patients of Tanaka *et al.* [23], lymphocytes and fibroblasts displayed 20–60 percent of control levels. Cultured fibroblasts of the neonatal patients incorporated significantly greater amounts of ^{35}S-labeled sodium sulfate into acid mucopolysaccharide than did those of cells of classic MSD patients [35]. Prenatal diagnosis has been reported [36] by sulfatase assay of cultured amniocytes or chorionic villus cells. In one patient with MSD, a low estriol was recorded in maternal urine [37], and in another with neonatal MSD, there was placental hormone deficiency [21]. Heterozygotes have been reported [38] to have intermediate activities of sulfatases in cultured fibroblasts.

As in MLD, urinary excretion of sulfatide is elevated. Sulfatide levels in the CSF are also increased. Accumulation of cholesterylsulfate has been identified in the liver, kidney, plasma, and urine. Cerebral gangliosides have been abnormal, as in Hunter disease [10, 18, 21].

The activity of sulfatases in cultured fibroblasts can be influenced by additions to the medium. Activity may be increased by the substitution of N-2-hydroxymethylpiperazine-N-2-sulfonic acid for bicarbonate buffer [34], and addition of leupeptin, a thiol protease inhibitor, leads to the appearance of arylsulfatase A activity and the ability to degrade labeled sulfatides [39]. These observations are consistent with the concept that the synthesis of the enzymes may be normal but their degradation is rapid [39]. Furthermore, isolated individual sulfatases from MSD material had normal kinetic properties [30, 34]. It was postulated that the mutation is an enzyme responsible for co- or post-translational modification of the sulfatase polypeptides [40]. Relevant to this hypothesis are the results of gene transfer of the cDNA for the arylsulfatases into fibroblasts [41]: cDNAs from MSD sources were expressed in normal and MSD cells. In MSD cells, mature enzyme proteins were present, but they were less than 5 percent as active as normal. This would fit with failure of an enzyme responsible for activation through modification.

In an elegant series of experiments, Schmidt *et al.* [5] showed that a cysteine predicted from the cDNA sequence

that is conserved among all known sulfatases is replaced by a 2-amino-3-oxopropionic acid in active enzymes, while in sulfatases of MSD cells, the cysteine is unchanged (see Figure 99.1). In arylsulfatase A, the cysteine of position 69 was found to be replaced with a compound containing no sulfur and an aldehyde function on tandem mass spectrometry, and the compound was definitely identified as 2-amino-3-oxopropionic acid in both arylsulfatase A and B. In MSD fibroblasts, the residue was cysteine. These observations have uncovered a novel modification of enzyme that confers catalytic activity on the protein. It is clearly the enzyme that catalyzes this modification that is defective in MSD.

As in the case of patients with MSD Sumf1(−/−) mice die early, and they have congenital growth impairment and skeletal and neurologic abnormalities. Vacuoles are visualized in histologic specimens, and there is significant storage of glycosaminoglycans in lysosomes. Macrophages were the predominant site of vacuolar lysosomal storage [42].

The gene *SUMF1* was cloned independently by Cosma *et al.* [7] and Dierks *et al.* [43] and mapped to chromosome 3p26 [43]. It contains nine exons spanning 105 kb. Some 30–50 mutations are known. Among mutations identified, a 4 bp deletion (GTAA) at position 5 of intron 3 leads to loss of the splice donor site for the intron and in-frame deletion of exon 3. It has been referred to as IVS 3 + 5-8 del [43] and 519 + 4 del GTAA [7]. One patient had a C-to-T transversion on the other allele at nucleotide 1076 (S359X) and another had R327Y on the other allele [7, 43]. In addition to a l bp deletion at 276C, missense mutations identified were: R345C, C218Y, A348P, and M1V [7]. Expression of the *SUMF1* genes carrying missense mutations revealed loss of function [9]. A compound heterozygote for two novel mutations (p.R349G and p.F244S) was reported in Brazilian patients [12]. A novel missense mutation c.739G > C causing a p.G247R amino acid substitution in the SUMF1 protein was reported in a Turkish family [44]. Four missense mutations p.A177P, p.W179S, p.A279V, and p.R349W did not affect localization of the FGE enzyme in the ER of MSD fibroblasts. The mutations did decrease the specific activity of the enzyme to less than 1 percent (p.A177P and p.R349W), 3 percent (p.W179S), or 23 percent (p.A279V). Protein stability was severely impaired in p.A279V and p.R349W, and almost comparable to wild type in p.A177P and p.W179S. The patient with the mildest clinical phenotype carried the mutation p.A279V leading to decreased FGE protein stability, but high residual enzymatic activity and only slightly reduced sulfatase activity. In contrast, the most severely affected patient carried the p.R349W mutation with drastically reduced protein stability, and residual enzymatic activity activities [45]. A patient with an infantile course of disease had two mutations c.156delC and c.390A > T [46]. The formula led to no detectable mRNA, but the latter localized correctly and had high molecular activity, but was very rapidly degraded. The division into distinct phenotypes is probably simplistic, and clear correlations between enzymes activity and clinical pictures are elusive [47].

Evidence of a generalized abnormality of T-cell development was found in multiple sulfatase-deficient mice [48]. In the knockout mouse model of MSD, there were enhanced apoptotic markers. Numbers of autophagosomes were increased compared to wild-type mice. This was thought to result from impaired autophagosome–lysosome fusion. The conceptualization of disorders of autophagy would fit with the conceptual idea of lysosomal diseases as neurodegenerative [49]. Autophagy is involved in recycling of proteins and organelles that follows fusion of autophagosomes with lysosomes. Lysosomal dysfunction impairs autophagy. Inactivation of SUMF1 and the accumulation of glycosaminoglycans in lysosomes led to disturbed autophagy [50].

TREATMENT

The symptomatic treatment of the leukodystrophy is supportive. Nasogastric or gastrostomy feeding may be required. Surgical fusion to stabilize the upper cervical spine may save the life of a patient or avert disabling quadriparesis. MRI is helpful in identifying candidates for surgery. Bone marrow transplantation has met with limited success in MLD and in various mucopolysaccharidoses. Experience is not available in MSD. Enzyme replacement therapy in mucopolysaccharidosis has encouraged the development of other enzymes for this purpose, including the sulfatases. The development of the expressed product of the *SUMF1* gene has potential for the treatment of individual sulfatase deficiencies, as well as of MSD [9].

The discovery of FGE as the sulfatase-activating enzyme has made the possibilities of enzyme replacement or gene therapy more logical [8]. Approaches to gene therapy have been explored in the *SUMF1* knockout mouse model, in which sulfatase activities are completely absent [51, 52]. Using a recombinant adeno-associated virus of serotype 9 (rAAV9 vector) encoding the *SUMF1* gene, it was found that combined intracerebral ventricular and systemic administration was superior to either single administration directly into brain, or systemic. The combined treatment led to widespread activation of sulfatases and virtually completes clearance of glycosaminoglycan in the central nervous system and viscera.

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PART 13

MISCELLANEOUS

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100

Disorders of vitamin B_6 metabolism

Pyridoxal-5′-phosphate (PLP) is an essential cofactor of transamination and decarboxylation reactions in various pathways, including biosynthesis of GABA, epinephrine, norepinephrine, serotonin and dopamine as well as degradation of glutamate and glycine [1]. It is synthesized from dietary pyridoxal, pyridoxamine, and pyridoxine (Figure 100.1). The conversion of pyridoxine and pyridoxamine to the only active cofactor, PLP, requires the activity of a kinase and then of pyridox(am)ine 5′-phosphate oxidase (PNPO); synthesis of the active cofactor from dietary pyridoxal or pyridoxal phosphate requires the kinase only. The three biologically active 2-methylpyridine derivatives collectively carry the generic name vitamin B_6. In the body, pyridoxine is found primarily in the liver and muscles. PLP is a highly reactive aldehyde, whose intracellular availability must be closely regulated to saturate all newly synthesized apo-enzymes while avoiding reactions to other nucleophiles. In humans, PLP deficiency causes peripheral neuritis, dermatitis, anemia, and relevant to neurotransmitter disorders, irritability, restlessness, hyperacusis, and convulsions in the central nervous system (CNS) [2].

Antiquitin (ATQ, ALDH7A1) (Figure 100.2) or α-aminoadipic semialdehyde dehydrogenase deficiency (pyridoxine-dependent epilepsy) [1, 3] and PNPO deficiency (pyridoxal-phosphate dependent epilepsy) [4] are two severe metabolic encephalopathies which respond to high dosages of pyridoxine and PLP. Their common pathophysiological denominator is deficiency of PLP, the active form of all B_6 vitamers.

In hypophosphatasia (see Chapter 105) and hyperphosphatasia, the transport of PLP across the cellular

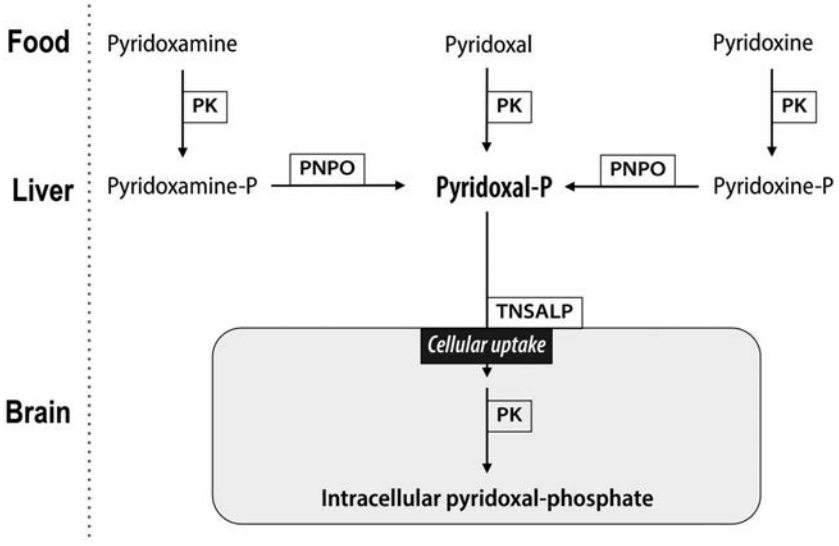

Figure 100.1 B_6 metabolism. Pyridoxal-5′-phosphate (PLP) is synthesized from dietary pyridoxamine, pyridoxal, and pyridoxine by pyridoxal kinase (PK) and pyridox(am)ine-5′-phosphate oxidase (PNPO). Membrane-bound tissue non-specific alkaline phosphatase (TNSALP) is involved in cellular uptake. In PNPO deficiency, the inability to produce sufficient amounts of pyridoxal out of pyridoxine and pyridoxamine, leads to insufficient amounts of PLP (adapted from [7]).

membrane is impaired, effectively resulting in intracellular PLP deficiency and, again, seizures. Although in these disorders seizures and intellectual disability can be part of the clinical picture, their major clinical manifestations include bone disease and symptoms of hypercalcemia (see Chapter 105; Figure 100.1; TNSALP deficiency). In hyperprolinemia type II, as in antiquitin deficiency [3], an intermediate accumulating because of the primary defect scavenges pyridoxal-phosphate leading to PLP deficiency (Figure 100.3). A similar mechanism is responsible for pyridoxal deficiency developing during treatment with the drugs isoniazid or penicillamine. In disorders involving the anchoring of glycosylphosphatidylinositol alkaline phosphatase cannot be anchored resulting in hyperphosphatasia and vitamin B_6 responsive epilepsy [5]. Finally, mutations in *PROSC* were recently identified to also cause vitamin B_6-dependent epilepsy because of disturbed intracellular PLP homeostasis [6]. Clinically, this disease presents in very similar fashion to PNPO deficiency (see Pyridoxal-phosphate dependent epilepsy [pyridox(am)ine-5′-phosphate oxidase deficiency] section below), with early and severe onset, often with fetal distress, untreated leading to severe acqured microcephaly and severe permanent handicap.

Antiquitin (ALDH7A1) mutations: Pyridoxine dependent epilepsy and folinic acid responsive seizures

MAJOR PHENOTYPIC EXPRESSION

Treatable neonatal seizure disorder, apnea, irritability, abnormal EEG, response to pyridoxine, folinic acid or both, elevated amounts of α-amino-adipic semialdehyde (α-AASA) and pipecolic acid, deficiency of α-aminoadipic semialdehyde dehydrogenase, and mutations in *ALDH7A1*, the antiquitin gene.

INTRODUCTION

Treatable neonatal epilepsy [8], exemplified by pyridoxine dependent epilepsy [1, 3, 8, 9] and folinic acid responsive seizures [10–12], has engaged attention because of early onset seizures unresponsive to the usual anticonvulsant medication, but impressively responsive to pyridoxine or folinic acid (formyltetrahydrofolate) or both. Patients may be recognized by assay of body fluids for α-AASA, pipecolic acid, and l-Δ1-piperideine-6-carboxylate, the latter being in equilibrium with AASA accumulating before the primary block (Figure 100.2).

It is now evident that pyridoxine dependent epilepsy and folinic acid responsive seizures are one disease, characterized by mutations in the antiquitin gene (*ALDH7A1)* which codes for α-aminoadipic semialdehyde dehydrogenase in the lysine pathway. Among 7 patients reported [13] 13 mutations were found. One mutation c.1208C > T, p.Pro403Leu was found on 3 alleles in 2 patients, and had previously been reported in pyridoxine dependent seizures [14].

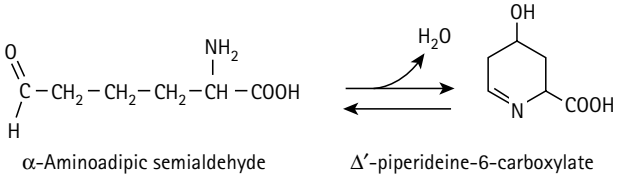

Figure 100.2 The α-aminoadipic semialdehyde dehydrogenase reaction, coded for by the Antiquitin gene.

CLINICAL ABNORMALITIES

Patients present usually in the first days of life with seizures [15–17] (Table 100.1). Late onset disease has been observed [16]. Seizures may be tonic, clonic, or myoclonic, and may lead to apnea. Twitching eye movements have also been described [13]. Convulsions at birth have been described [17], and even *in utero* [17]. Birth is often complicated and one third of children present with asphyxia. Abnormal Apgar scores and cord blood gases may also be observed, and it is not uncommon for these newborns to be diagnosed hypoxic ischemic encephalopathy. Presentation with status epilepticus is common. Death in status epilepticus has been reported [17]. Seizures are typically unresponsive to the usual anticonvulsant medications, but some have had a good initial response to first line anti-epileptic drugs such as phenobarbitone or phenytoin [18]. Patients may be irritable or restless, and vomiting may occur. Other non-convulsive symptoms indicate that pyridoxine dependent epilepsy is an encephalopathy with epilepsy as the most dominant symptom.

Later onset, as well as pyridoxine responsiveness was reported [18] in three patients who could be seizure free for several months after cessation of pyridoxine. The onset of the disease in some patients well beyond infancy has led to the recommendation that this disease should be tested for in any child with intractable epilepsy, at least up to three years. This is especially true in a family in which more than one patient had intractable seizures [3]. A few patients did not or only partly respond to pyridoxine, but responded to folinic acid [10–13]. One patient we care for

Table 100.1 Hints to pyridoxine-dependent and pyridoxal-phosphate-dependent epilepsies

Family	Patient
Consanguineous parents	Unusual fetal movements
Miscarriage(s)	Prematurity, fetal distress, "signs of asphyxia"
Stillbirth(s)	and low Apgar scores
Previous sibling with severe seizures or	Irritability, hyperexcitability, and vomiting
Death in status epilepticus	Prolonged, recurrent seizures refractory to standard antiepileptic drugs

[10] initially responded only to folic acid and to pyridoxine from the age of three years and has remained with good control and satisfactory outcome since for over 20 years. Developmental delay ranging from mild to severe has been observed, and most patients have some cognitive impairment [17, 19]. Adult neurologists should be aware that the diagnosis of pyridoxine-dependent epilepsy can be delayed and should be considered in the differential diagnosis of adults with seizure disorders dating from childhood. In a 19-year-old patient with antecedents of neonatal seizures associated with shunted hydrocephalus and refractory status epilepticus, pyridoxine-dependent seizures was genetically confirmed [20, 21].

Patients with pyridoxine dependency often do not have a characteristic EEG pattern such as high-voltage; bilaterally synchronous 1- to 4-Hz EEG activity occurring in bursts and associated with intermixed spikes and sharp waves. This picture and even the alterations by IV pyridoxine such as attenuation of seizure activity and flattening of the trace can be absent in proven antiquitin deficiency, but a positive response may be seen in an infant with an epileptic encephalopathy of different etiology [22]. It is, therefore, of utmost importance to confirm the suspected diagnosis of antiquitin deficiency by biochemical and/or genetic analyses. It is critical for the individual prognosis, lifelong potential of response to pyridoxine even in initially non-responsive patients, the possibility of treating the mother in case of future pregnancies, and the avoidance of potential side effects of prolonged pyridoxine substitution in biochemical and genetic negative patients.

There are no specific imaging findings in antiquitin deficiency. Reports on cerebral imaging demonstrated progressive ventricular dilation and varying degrees of atrophy of the cortex and subcortical white matter especially in late-diagnosed patients. There can also be cerebral hemorrhage, thinning of the corpus callosum, particularly the posterior region, white matter lesions, cerebellar hypoplasia, cortical dysplasia, neuronal migration abnormalities, and a megacisterna magna [17]. Neuronal migration abnormalities and progressive hydrocephalus requiring shunting have been reported in several patients. The demonstration of structural brain abnormality by NMR should therefore not preclude investigations for antiquitin deficiency.

α-AASA is elevated in blood, urine, and cerebrospinal fluid (CSF) [19, 23]. Concentrations in plasma have ranged from 0.9–8.0 μmol/L and in urine from 4 to 75 mmol/mol creatinine [23]. The availability of this biomarker for the disease has made clinical testing for pyridoxine responsiveness obsolete, however empirical treatment with pyridoxine should not be withheld until diagnostic samples are collected. This concept is particularly useful for it makes the withdrawal of treatment unnecessary to pursue testing. Pipecolic acid is also elevated in plasma and CSF, but this may be only modestly elevated, and this is a less reliable biomarker. Pipecolic acid is also elevated in other inborn errors of metabolism such as disorders of peroxisomal biogenesis, and hyperlysinemia. Urinary levels of pipecolic acid are often normal [23]. Levels of piperideine-6-carboxylate are also elevated; this compound is in equilibrium with α-AASA (Figure 100.2) in the pathway for the catabolism of lysine. α-AASA is also elevated in molybdenum cofactor deficiency [24], another cause of severe neonatal seizures. Levels of excretion range from 9.6–16.9 mmol/mol creatinine, certainly within range of antiquitin deficiency. Hypouricemia or elevated excretion of xanthine, sulfite or sulfocysteine in molybdenum cofactor deficiency should elucidate the differential diagnosis.

Neonatal seizures and pyridoxine dependency have also been reported [25] in PNPO deficiency (see Pyridoxal-phosphate dependent epilepsy [pyridox(am)ine-5′-phosphate oxidase deficiency] section below). Reference values have been established for PLP in CSF and plasma [5, 25]. They were stratified according to a negative correlation with levels and age. In controls, the median levels were 52 nmol/L in the under 30 day group (range 32–78), 43 in 1–2 months (24–87), 31 in 1–2 yrs (14–59) and 21 in 3–19 yrs. In four patients with PNPO deficiency levels were clearly below reference values.

GENETICS AND PATHOGENESIS

The disease is transmitted in an autosomal recessive fashion. Its incidence was estimated at 1 in 276,000 newborns in the Netherlands [23] and up to 1 per 700,000 births in other countries [26].

Accumulated piperideine-6-carboxylate reacts with pyridoxal-5-phosphate in a Knoevenagel condensation (Figure 100.3a). This is an irreversible reaction which depletes PLP and results in seizures treatable by oral pyridoxine which resolves the seizure disorder. Hyperprolinemia (type II) due to defective activity of Δ^1 pyrroline-5-carboxylate dehydrogenase leads to the accumulation of P5C

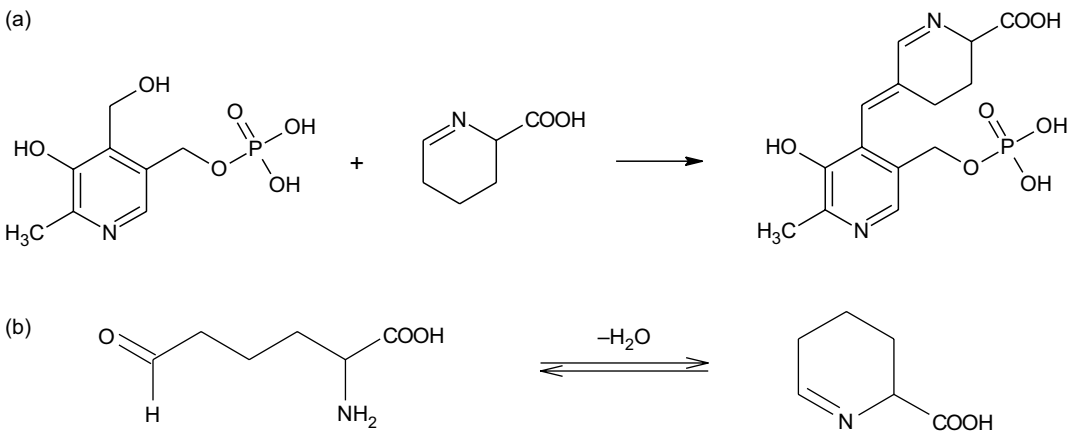

Figure 100.3 (a) Knoevenagel reaction. PLP (right) spontaneously reacts with accumulating L-Δ1-piperideine-6-carboxylate (middle). The complex (left) formation is irreversible depleting PLP. (b) Spontaneous conversion between alpha-aminoadipic semialdehyde (right) and L-Δ1-piperideine-6-carboxylate (left).

which also reacts with PLP by a Knoevenagel condensation and is characterized by seizures. This provided the clue [19] to the pathogenesis of pyridoxine-dependent seizures and the defective mutation in antiquitin.

The gene for antiquitin *ALDH7A1* was mapped to chromosome 5q31, the locus already reported [27] for pyridoxine dependent seizures. ALDA7A1 was sequenced in material from 18 patients with pyridoxine dependent seizures [19]. Homozygous and compound heterozygous mutations were found in all 18 families, and parents were found to be heterozygous. Ten novel mutations were found, one a deletion of exon 7. A known mutation, c.1195G > C; pGlu399Gln, in exon 14 was found to be relatively prevalent, accounting for 12 of 36 alleles (33% of this cohort). To date, close to 100 mutations have been reported within the 18 exons of *ALDH7A1*, 50–60 percent being missense mutations. A high number of missense mutations cluster around exons 14, 15, and 16.

In two families splice site mutations, c.228 + 2t > a and c.434-1g > c, were predicted to cause exon skipping [19]. The latter would skip exon 6, leading to a protein lacking amino acids 145–189, including amino acids found in more than 95 percent of antiquitin enzymes. A missense E399Q affects a glutamate highly conserved in antiquitin. A number of nonsense codons were found: R82X would lack residues 82-511 in the enzyme protein. An intriguing "silent" mutation (c.750G > A) was identified in to touch families. Although this type of DNA variation is usually considered nonpathogenic, this variant proved to be pathogenic because of erroneous splicing [28].

TREATMENT

Experience has indicated that treatment of these patients with pyridoxine is prudent. There is no universal protocol for a pyridoxine trial. The dose required is variable, and higher doses may be necessary to control seizures, at least initially. In classical cases, we suggest a starting dose of 100 mg intravenously. If there is no response within 24 hours, the dose should be repeated (and possibly increased up to 500 mg in total) before being sure about pyridoxine nonresponsiveness. If there is uncertainty about at least a partial response, pyridoxine should be continued at 30 mg/kg/day for seven days before final conclusions are drawn. In case of no response a trial of folinic acid (3–5 mg/kg of body weight per day in three doses for three days) should be given, and finally pyridoxal 5′-phosphate (30 mg/kg of body weight per day in three doses again for three days). A variety of different doses has been explored for maintenance; 15 mg/kg was effective in many but responsiveness has been observed with as little as 0.5 mg/kg/day [18]. Obvious criteria to increase the doses are breakthrough seizures. Patients require lifelong supplementation. In brittle epilepsy an add-on of folinic acid (3–5 mg/kg/d) can be tried [13]. In patients with incomplete seizure control, additional pharmacologic anticonvulsants may be required. During an acute illness, such as gastroenteritis or a febrile respiratory infection, patients may have exacerbations of the seizures or encephalopathy. In these circumstances, the daily dose of pyridoxine can be doubled for several days until the acute illness resolves. The demand for pyridoxine is increased during pregnancy. Status epilepticus occurred during pregnancy in a woman treated with pyridoxine-dependent epilepsy from early childhood, who had been seizure free for 23 years with oral pyridoxine supplementation. Seizures were again unresponsive to antiepileptic medication but stopped with parenteral administration of vitamin B_6 [21].

Pyridoxine neuropathy provides a limiting factor in the escalation of dosage, but this has been observed only in those receiving over 500 mg per day [18].

While treatment with pyridoxine compensates chemical PLP inactivation, the accumulation of substrates from lysine degradation is not reduced. These potentially neurotoxic compounds could be an explanation of the limited efficacy of pyridoxine treatment although the

outcome appears even worse in PNPO deficiency with no metabolites accumulating. 70–75 percent of patients suffer from developmental delay or intellectual disability (IQ < 85) despite excellent seizure control in the majority of patients [17]. Dietary lysine restriction in addition to pyridoxine therapy was well tolerated with significant decrease of potentially neurotoxic biomarkers and with the potential to improve developmental outcomes and seizure control in children with antiquin deficiency [29]. Arginine fortification has proven safe as an add-on treatment to lysine restriction [30].

Antenatal treatment of affected fetuses with supplemental pyridoxine (50–60 mg/day) given to the mother during pregnancy has proven effective in preventing intrauterine and neonatal onset seizures and improved outcome as compared to the index sibling in single cases [31].

Pyridoxal-phosphate dependent epilepsy (pyridox(am)ine-5′-phosphate oxidase deficiency)

MAJOR PHENOTYPIC EXPRESSION

Prematurity, fetal distress and neonatal presentation with convulsions, myoclonus, rotatory eye movements, sudden clonic contractions, hypoglycemia, and (lactic) acidosis only responsive to PLP. Elevated amounts of glycine and biochemical mimicry of aromatic L-amino acid decarboxylase deficiency. Deficiency of pyridox(am)ine-5′-phosphate oxidase (PNPO), and mutations in the *PNPO* gene.

INTRODUCTION

Pyridoxal-5′-phosphate (PLP) plays numerous roles in over 140 metabolic reactions, including biosynthesis or degradation of all neurotransmitters [1]. It is synthesized from dietary pyridoxal, pyridoxamine, and pyridoxine (see Figure 100.1). The conversion of pyridoxine and pyridoxamine to the only active cofactor, PLP, requires the activity of a kinase and then of PNPO; synthesis of the active cofactor from dietary pyridoxal or pyridoxal phosphate requires the kinase only, i.e. bypassing PNPO. PNPO probably also plays a role in a salvage pathway recycling the cofactor from degraded enzymes.

CLINICAL ABNORMALITIES

Almost all PNPO-deficient patients have a severe and acute early neonatal presentation with convulsions, myoclonus, rotatory eye movements, and sudden clonic contractions [4, 7]. It is important to note that many but not all patients are born prematurely between 22 and 35 weeks' gestation. Fetal distress is common, as are "signs of asphyxia". Most have had a low Apgar score and/or required intubation. Thus, PNPO deficiency must enter the differential diagnosis of hypoxic–ischemic encephalopathy (HIE) in a prematurely born infant. Seizures are resistant to conventional anticonvulsant therapy and can be fatal. EEG shows a burst-suppression pattern. There is often metabolic (lactic) acidosis as well as a tendency to hypoglycemia. There may be gastrointestinal problems such as abdominal distension, even ileus and vomiting. Very similar is the presentation of babies affected with the vitamin B_6-dependent epilepsy caused by mutations in *PROSC* [6].

We frequently experienced family histories of previously affected siblings which almost invariably succumbed in the first days or months of life to their epilepsy, often combined with gastrointestinal problems and severe failure to thrive [32, 33]. One family lost six affected children and were instrumental in finally identifying the defect [4, 32]. Their first child had been born at 32 weeks gestation and died at 21 days. This infant was thought to have non-ketotic hyperglycinemia on the basis of epileptic encephalopathy plus elevated plasma and CSF glycine but the ratio was normal. The next five children were born even more prematurely. Two died at day one, three survived for 17, 19 days, and 6 months, respectively. From birth, all had severe convulsions, myoclonus, rotatory eye movements, and sudden clonic seizures. In retrospect, treatment with pyridoxine led to a partial improvement of clonic contractions and lip-smacking automatisms. The longest living girl developed progressive microcephaly and died at six months of her epilepsy plus symptoms of ileus and severe failure to thrive (1.56 kg). PNOP deficiency was only then confirmed. Tragically, in the next pregnancy DNA analysis from chorionic villus indicated that this fetus was again affected (homozygous for the R229W mutation), and the pregnancy was terminated. Another family had again lost two children, when PNPO deficiency was diagnosed in a boy who had survived for three and a half years despite severe epilepsy which persisted under various antiepileptic drug regimens including ketogenic diet and ACTH [33]. His psychomotor development was severely retarded. Awake he was constantly seizing, his weight at this age was 6.4 kg, and his head circumference 44 cm. All three affected children of this family had initially presented with lactic acidosis. Remarkably, detailed metabolic investigations including CSF analyses performed during infancy did not reveal any of the abnormalities reported

as characteristic for PNPO deficiency, but 23 minutes after the first oral administration of PLP the EEG burst-suppression pattern changed to complete suppression for 90 minutes before alpha- and theta activity resumed. PLP treatment from then on resulted in markedly reduced seizure activity, albeit seizures did not stop completely. Further improvement could be reached by increasing dose and frequency of daily administrations, as seizures became more frequent at the end of a six hour drug-free interval. Over the next three months, the boy became more alert and reactive; the gastrointestinal problems subsided, and his weight increased by 50 percent.

With more patients known, it became obvious that the clinical presentation of PNPO deficiency and antiquitin deficiency were overlapping if not identical [4, 7, 33, 34]. Patients suffering from PNPO deficiency tend to present with a more severe and complex symptomatology. Clinical responsiveness to pyridoxine does not exclude PNPO deficiency. Thus, patients with pyridoxine dependency negative for antiquitin deficiency should be tested for PNPO deficiency and if negative for mutations in *PROSC*. PNP administered by nasogastric tube is mostly dramatically effective in stopping the seizures and improving the EEG. First administration may result in respiratory arrest in responders, and thus treatment should be performed with support of respiratory management [4]. Patients with PNPO deficiency usually require more frequent and equally spaced administration of PNP than patients with antiquitin deficiency, e.g. every three to four hours. This can be guided well by EEG monitoring which normal at first, shows generalized rhythmic fast activity. After PLP symptoms resolve within minutes, and the EEG normalizes. Cranial MR is mostly normal early in the disease course or shows unspecific findings of encephalopathy (Figure 100.4). Later, MRI scans may reveal severe diffuse cerebral atrophy in untreated or only partially treated patients.

PNPO deficiency may have a complex biochemical phenotype which again has been at least partially reported in antiquitin deficiency, but characteristic biochemical findings may be missing [7]. The most characteristic changes may be found by CSF analysis with a biochemical pattern indicative of aromatic L-amino acid decarboxylase (AADC) deficiency (see Chapter 17). Concentrations of HVA, 5-HIAA, and MHPG are all decreased in association with increased concentrations of L-DOPA, 5-hydroxytryptophan, and 3-OMD. In urine, vanillactic acid can be found elevated by organic acid analysis (Figure 100.5). The elevation of vanillactic acid in urine detectable by organic acid analysis can help in pinpointing the disorder. However, elevations are mostly slight, and laboratories will not automatically search for this compound unless specifically instructed. Activity of AADC in plasma is, however, normal or even increased. Additional biochemical abnormalities in most patients published included raised CSF levels of lactate, glycine, threonine, taurine, histidine, and low arginine. The analysis of vitameric B_6 forms in plasma reveals decreased

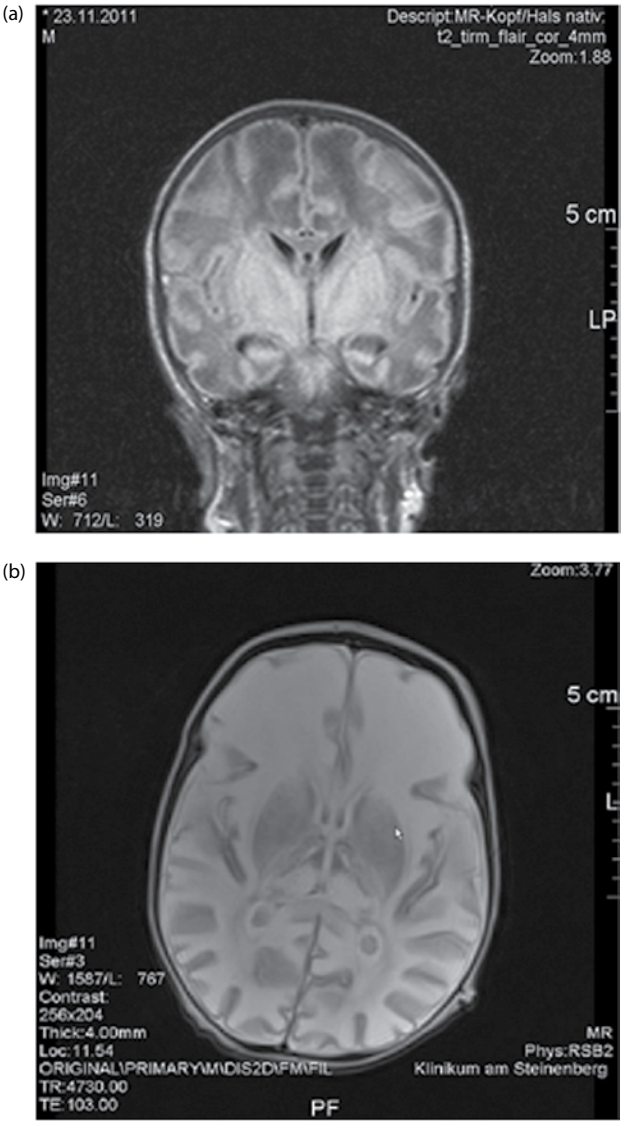

Figure 100.4 Cerebral imaging of a full-term baby who presented 10 minutes post-partum with respiratory distress and abnormal posturing. Status epilepticus developed which initially did not respond to antiepileptic drugs including pyridoxine and PLP. In the second week of life, epilepsy could be controlled with pyridoxine, PLP and folinic acid. Please note swollen white matter (a) as well as disturbed diffusion in the cortex (b). Because mutation analysis of the PNPO gene proved initially negative, PLP was discontinued at three months of age, resulting in immediate reoccurrence of seizures 4.5 hours later. PLP was reintroduced and seizures stopped immediately. A homozygous mutation was finally proven a few months later. (Courtesy of Dr. A Keil, Witten, Germany.)

PLP values in CSF of affected neonates but is, as yet, only available on a research basis [25, 35]. A characteristic pattern with distinct elevations of pyridoxamine, pyridoxamine-phosphate, pyridoxine, pyridoxine-phosphate and low PLP was found in two patients with genetically proven PNPO deficiency on treatment with 30 mg/kg/d PLP (see Figure 100.1) [35].

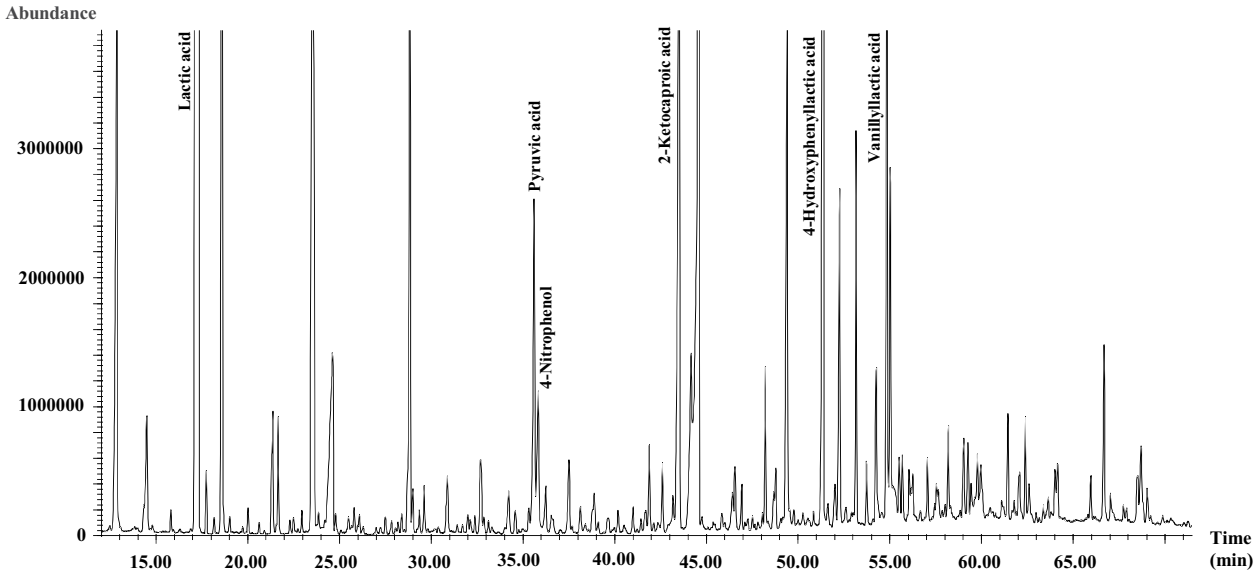

Figure 100.5 Urinary organic acids chromatogram of a premature newborn suffering from PNPO deficiency. There is a striking elevation of VLA, which is undetectable in controls and usually much lower in other patients and may then be even missed (adapted from [32]).

GENETICS AND PATHOGENESIS

Pyridoxal-phosphate dependent epilepsy due to PNPO deficiency was originally delineated in three families with five (by family history nine) children [4, 32]. Mutations identified by now, about 20, are mainly missense with different levels of functional impairment which potentially explain the various clinical response patterns to PLP. Mutations affecting the binding site of pyridoxine might explain why some patients respond to pyridoxine [34, 36].

PNPO catalysis the synthesis of PLP from pyridoxine and pyridoxamine (see Figure 100.1). It requires flavin mononucleotide (riboflavin-5′-phosphate) as a tightly bound (prosthetic) cofactor. Because of the inability to produce sufficient amounts of pyridoxal out of pyridoxine and pyridoxamine, pyridoxal phosphate becomes the only source of the active cofactor. This leads to impairment of aromatic L-amino acid decarboxylase, threonine dehydratase, ornithine δ-aminotransferase, and the glycine cleavage enzyme. In addition, mitochondrial energy metabolism can be assumed to be compromised because the mitochondrial aspartate shuttle that transports reducing equivalents to the mitochondria requires pyridoxal-phosphate dependent aspartate aminotransferases in the cytosol as well as in the mitochondria. This may explain elevated lactate levels in blood and CSF noted in affected patients. Furthermore, glycogenolysis likely is reduced because glycogen phosphorylase uses PLP as a cofactor. Additionally, conversion of lactate to glucose during fasting may be impaired because in the fasting state the citric acid cycle in the liver is supplied by the carbon skeletons of glucogenic amino acids, and these are produced by PLP-dependent reactions. Both mechanisms can disturb glucose homeostasis and explain the clinically observed hypoglycemia.

TREATMENT

Successful treatment of pyridoxal-phosphate dependent epilepsy is achieved with doses of 10–80 mg PLP per kilogram of body weight per day in four to five doses [4, 7, 34]. Doses need to be increased and adjusted to body weight during growth. Hepatotoxicity is a possible dose-dependent side effect. Patients require lifelong supplementation. Obvious criteria to increase the doses are breakthrough seizures. Pyridoxine and folinic acid are further options to consider in case of suboptimal response.

In only a few patients so far, specific therapy has been instituted soon after onset of symptoms. Some treated with PLP within the first month of life showed normal development thereafter [7, 34]. Most patients are known have significant developmental delay but remain in a stable condition. With regard to treatment and prognosis, many questions are open. In one patient, an autopsy revealed brain damage that was probably prenatal in onset [4], raising the perspective of antenatal treatment as already undertaken in antiquitin deficiency [31].

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101

PMM2-CDG (Congenital disorders of glycosylation, type Ia)

MAJOR PHENOTYPIC EXPRESSION

Infantile failure to thrive, inability to aliment orally, developmental delay, hypotonia, inverted nipples, esotropia, and an unusual lipodystrophy in which a general decrease in subcutaneous fat is associated with accumulated large fat pads in unusual sites, such as above the buttocks; pericardial effusions, hepatic dysfunction and pontocerebellar hypoplasia; in childhood, ataxia and disequilibrium, retinitis pigmentosa and stroke-like episodes; teenage neuropathy, muscular atrophy and secondary skeletal deformities; adult hypogonadism; deficient or absent carbohydrate moieties of secretory glycoproteins, especially serum transferrin; and deficient activity of phosphomannomutase.

INTRODUCTION

Twin girls with impaired psychomotor development and strabismus were reported in 1980 by Jaeken and colleagues [1] to have decreased amounts of thyroxin-binding globulin (TBG) in the serum and increased activity of serum arylsulfatase A. It was recognized that this unusual association was with two quite different glycoproteins, and heterogeneity was then found in transferrin of serum and cerebrospinal fluid (CSF) [2]. It was hypothesized that the defect in common was in the carbohydrate moiety. Confirmatory evidence was the demonstration of deficiency of sialic acid, galactose, and *N*-acetylglucosamine in transferrin and in several other serum glycoproteins [3]. In 1995, the molecular cause was published, deficiency of phosphomannomutase 2 (PMM2-CDG) (EC 5.4.2.28) (Figure 101.1) [4, 5].

More than 30 patients had been reported by 1993 [6] and more than twice that number had been identified in Scandinavia alone [7]. By now more than 1000 patients have been identified worldwide. The disorder was originally described in Belgium [1], but patients have been recognized in Spain [8], Taiwan [9], and the United States [10], including some of African ancestry [11].

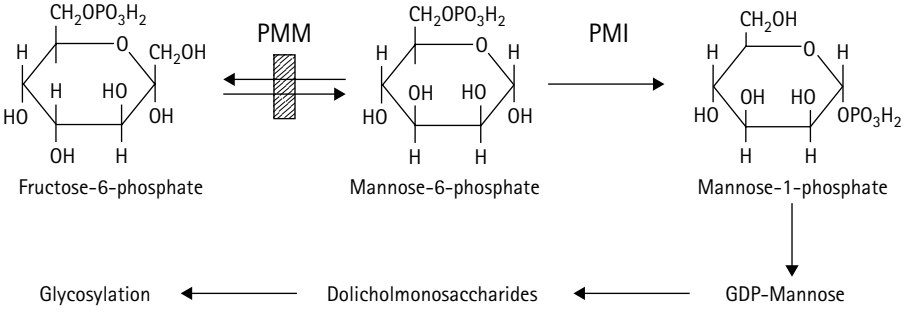

Figure 101.1 The phosphomannomutase 2 reactions (PMM) and phosphomannose isomerase (PMI) and its relation to glycosylation.

Glycosylation comprises all processes in which carbohydrates are added to proteins and lipids, thereby modifying their properties. It involves a huge variety of enzymes involved in the synthesis and processing of nucleotide-activated sugars, vesicular transport and glycosylation of biomolecules directing a tremendous heterogeneity of physiologic functions. Following PMM2-CDG, related disorders of glycosylation have been discovered. By now, more than 60 entities comprise a still growing group of monogenetic diseases of glycoprotein biosynthesis termed congenital disorders of glycosylation (CDG). The oligosaccharide moieties determine critical biologic processes like protein quality control, directed protein transport, enzymatic activity, and protein stability. Deficiencies lead to multiorgan diseases with neurologic symptoms often dominating. The identification of a considerable number of "new" CDG-types afforded the improvement of nomenclature which now connects the abbreviation of defective proteins with the term CDG (e.g. deficiency of the enzyme phosphomannomutase 2 (PMM2), formerly known as CDG-Ia, changed to PMM2-CDG.

CLINICAL ABNORMALITIES

Different phenotypic manifestations characterize this disorder at different ages. They have been differentiated [6] as:

I. Infantile multisystem stage;
II. Childhood ataxia-mental impairment stage;
III. Teenage leg atrophy stage;
IV. Adult hypogonadal stage.

This classification not only characterizes the clinical manifestations at different ages, but also reflects the severity of disease from fatal hydrops fetalis at the severe end [12] to a mild neurologic phenotype in adults even with normal cognition [13].

Most patients have been born at term after an uneventful pregnancy, and birthweight has usually been normal. Some features may be recognized at birth: inversion of the nipples (Figures 101.2 and 101.3) is nearly universal (Table 101.1) and so is esotropia [6, 7]. Lipoatrophy may be manifest in a general reduction of subcutaneous tissue mass [7], or in lipoatrophic streaks or patches [6]. The most unusual feature is the occurrence of fat pads – collections of subcutaneous tissue in unusual places, most typically over or above the buttocks, but elsewhere too (Figures 101.4–101.8). Patches of thickened skin, especially on the legs, have been described as like tallow or peau d'orange (orange peel) [6]. These features may be absent in the early months, in which the only manifestation may be failure to thrive. Alternatively, there may be early neonatal hypotonia, lethargy, edema, or cardiac failure [12]. Prolonged hypoglycemia has also been described as the first symptom of PMM2-CDG [14].

Difficulties with feeding and failure to thrive regularly characterize the first three months. These infants display little interest in nursing, and nasogastric feedings have been required throughout the first year [7]. Frequent and projectile vomiting has been a problem. At one year, most patients have just doubled the weight of birth. Linear growth is also behind. Even older children may not chew, and they may gag and choke on textured or lumpy foods. However, notable exceptions have been observed, and macrosomia may be found [15].

Dysmorphic facial features described have included bitemporal narrowing, a broad nasal bridge, prominent jaw, full cheeks, and large external ears (Figure 101.9) [5].

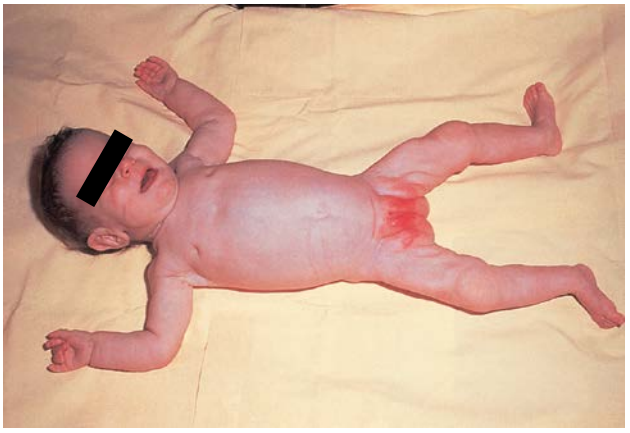

Figure 101.2 HS: An eight-month-old infant with carbohydrate-deficient glycoprotein disease. Illustrated are the inverted nipples that constitute an early sign of the syndrome. There were lipoatrophic changes of the lower extremities. (This and the other pictures of patients with this disease were kindly provided by Dr J Jaeken of the University of Louven, Belgium.)

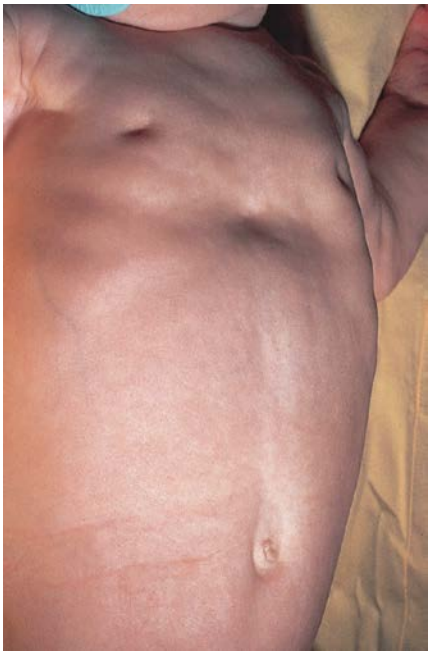

Figure 101.3 HS: At eight months, showing close up of the inverted nipples.

Table 101.1 Inverted nipples: differential diagnosis

Congenital disorders of glycosylation
Biopterin synthesis disorders (Chapter 16)
Citrullinemia (Chapter 28)
Isolated autosomal dominant (McKusick No. 163600)
Menkes disease
Methylmalonic acidemia (Chapter 3)
Molybdenum cofactor deficiency
Propionic acidemia (Chapter 2)
Pyruvate carboxylase deficiency (Chapter 48)
Very long chain acyl coenzyme A dehydrogenase deficiency (Chapter 40)
Weaver syndrome

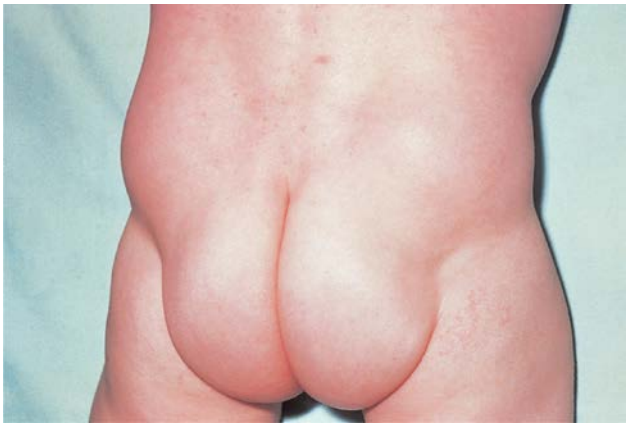

Figure 101.6 SF: This figure shows a close up of the fat pads.

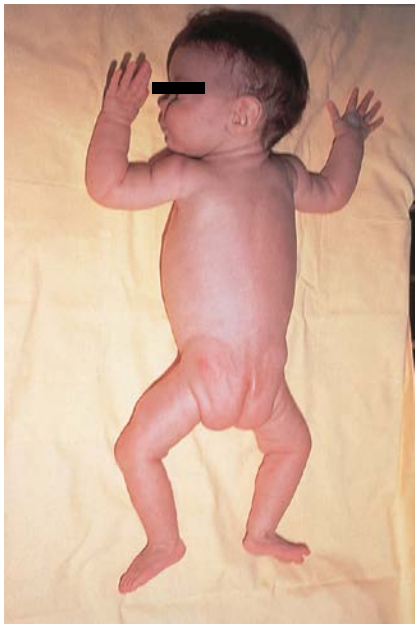

Figure 101.4 HS: This figure illustrates the characteristic fat pads on the buttocks.

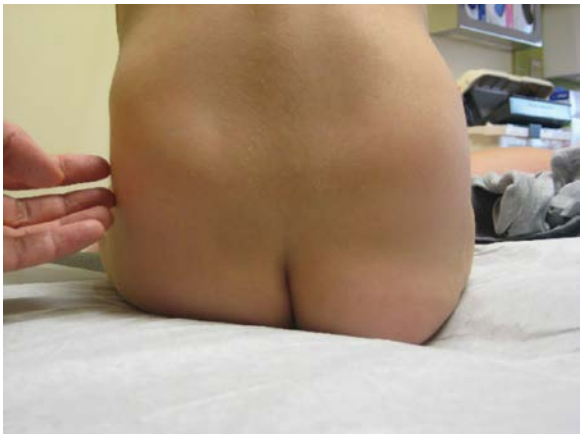

Figure 101.7 Fat deposits above the buttocks of a patient at 18 months.

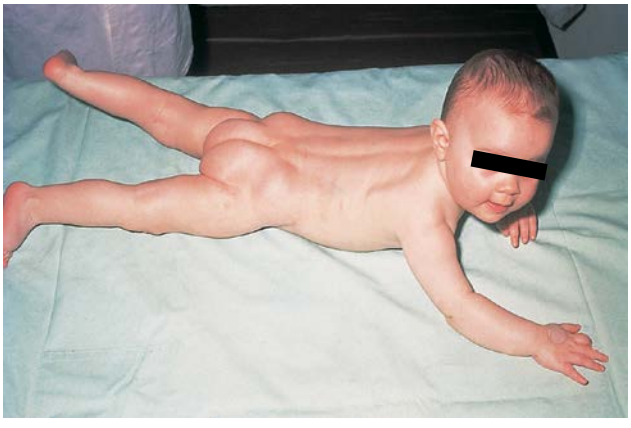

Figure 101.5 SF: A nine-month-old patient with the characteristic fat pads over the buttocks.

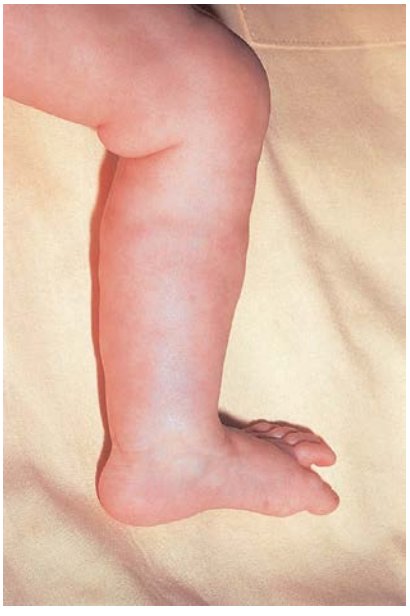

Figure 101.8 HS: At eight months illustrating that fat deposits may occur elsewhere.

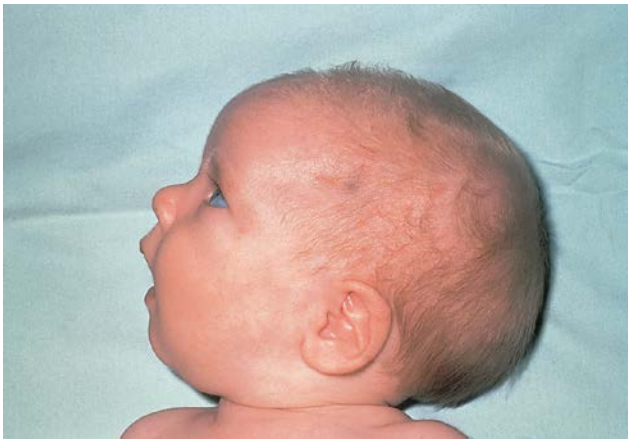

Figure 101.9 LA: At two months. The face was somewhat dysmorphic, the forehead prominent, the nasal bridge depressed and tip anteverted, and there was some micrognathia. The ears were relatively large.

Some have had limitation of joint mobility in the legs. Head circumference at birth is normal, but microcephaly develops in about half of the patients [6].

Hypotonia or floppiness is regularly observed, and developmental delay is obvious early. Head lag may be seen as late as 12 months. In addition, some infants have had stroke-like episodes or episodes of acute deterioration in which developmental landmarks achieved have been lost. A few have had seizures. Ataxia is recognizable as early as seven months and imaging of the central nervous system reveals cerebellar and pontine atrophy, which may appear, especially after episodes of acute exacerbation (Figure 101.10). A reduction of nerve conduction velocity, especially at the lower limbs, may manifest at the age of 6 to 8 months. Disappearance of tendon reflexes has been observed by one year of age. After infancy, retinitis pigmentosa develops. Hepatomegaly is a regular feature. Blood levels of transaminases are increased and levels of albumin and coagulation factors are decreased. There may be intestinal bleeding. Enlarged kidneys may be demonstrated by ultrasound or other forms of imaging, and nephrotic syndrome may occur due to multiple microcysts.

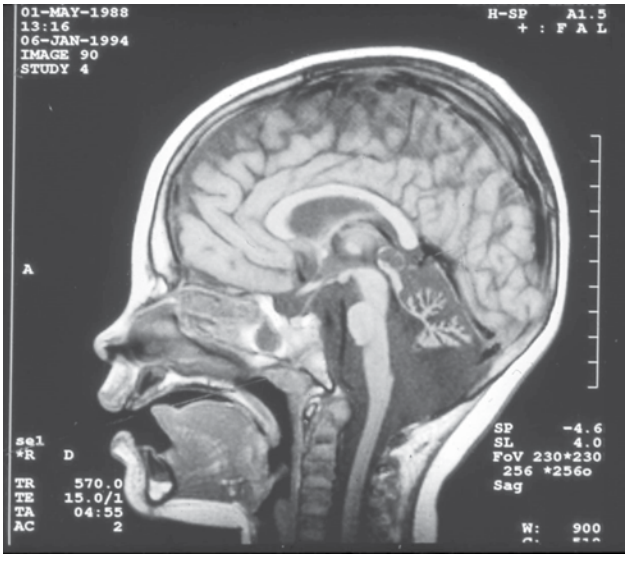

Figure 101.10 Sagittal NMR of a five-year-old patient with PMM2-CDG illustrating cerebellar and pontine atrophy.

Recurrent episodes of pericardial effusion have been seen commonly in infancy, and death from cardiac tamponade has been recorded [6]. There may be cardiomyopathy. Serious infections are also common, and an infantile and early childhood mortality of 15–20 percent reflects predominantly infectious disease.

The childhood period from three to 12 years of age [5, 13, 15] is characterized by ataxia and mental impairment (Figure 101.11). Some have dyskinetic or choreoathetotic episodes. Only a few patients have learned to walk. Most sit unsupported after two years; they ultimately learn to stand on tiptoe because of contractures [16]. Most learn to use a wheelchair. Disequilibrium and impaired coordination are prominent. Motor impairment is uniformly seen but the degree of impaired mental development is variable: IQs have ranged from 40 to 60 [6]. Patients understand spoken words but few develop linguistic skills; they speak in staccato fashion. Intellectual regression has not been seen except following stroke-like episodes. Deep tendon reflexes in the lower extremities disappear at this stage, and peripheral neuropathy becomes evident [16]. Retinal degeneration and retinitis pigmentosa is progressive

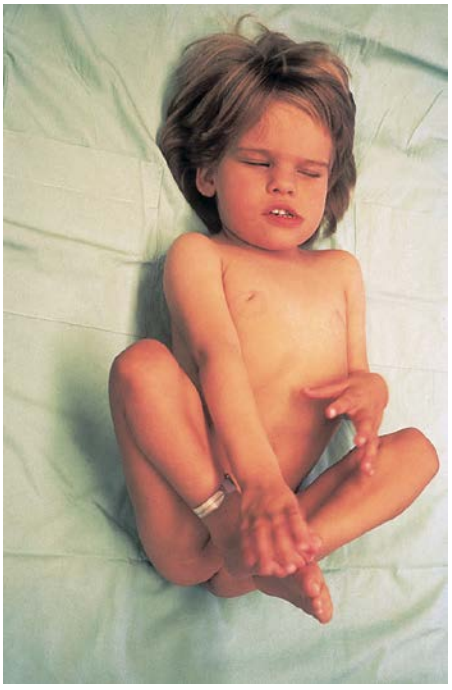

Figure 101.11 LA: A three-year-old female, was developmentally impaired and had motor disability of the lower limbs. Posturing of the left hand is evident. Retinitis pigmentosa leads to loss of vision.

in most [17]. Defective hemostasis, due to the reduced amounts of factor XI and the anticoagulation factors protein C and antithrombin III, leads to thromboembolic complications. Stroke like episodes occur often during pyretic infections. The stroke-like episodes are more prominent in childhood; there may be stupor or coma, and convulsions, as well as hemiplegia, usually with recovery in hours to days. Permanent hemiplegia has been associated with cerebral infarction. Two patients were blind for months after an episode. One patient had an arterial thrombosis in a hand [18].

The teenage years are dominated by progressive muscle atrophy and weakness, especially of the legs. This appears primarily to be due to lower motor neuron dysfunction. Nerve conduction velocity is reduced. Cerebellar ataxia and poor coordination continue. Skeletal deformities, kyphosis, scoliosis, and keel thorax appear to be consequences of muscle atrophy. The unusual fat pads may disappear during this period. Seizures occur in about 50 percent of patients, but frequency may decrease in adolescence. Hepatopathy may stabilize or disappear.

Hypogonadism may be recognized in this period or in adulthood. This appears especially common in females. It may be hypergonadotrophic [6], but testicular atrophy has been seen in a male. There may be intermittent elevation of prolactin, growth hormone, insulin or follicle stimulating hormone (FSH). The lack of pubertal development and primary ovarian failure in CDG females resembles the phenotype in galactosemia. Premature ageing may also be seen in young adults [6]. Adults are short, compressed, and bent into flexor deformities. Thoracic and spinal deformities progress. Neurologic deficits seem to stabilize in adulthood. Milder affected patients have been diagnosed in adulthood with cerebellar ataxia [13, 19]. Multisystem involvement is mostly present but intelligence maybe normal [13].

Clinical laboratory evaluation may reveal proteinuria. There is often an intermittent thrombocytosis, with counts up to 800,000 per mm^3. There may be hypoprothrombinemia and diminished factors IX and XI. Elevated transaminases typically normalize after the first two years of life but become elevated during illness. Serum albumin is usually low, and some have a hypo-β-lipoproteinemia. The stroke-like episodes and thrombotic disease have been associated with decreased levels of protein C and antithrombin III and other major inhibitors of coagulation [18]. Thyroxine-binding globulin (TBG) is reduced in 75 percent of patients. CSF protein may be elevated. In general, patients with CDG are clinically euthyroid but may present with the biochemical picture of partial TGB deficiency (low total T_4, normal free T_4, normal thyroid-stimulating hormone [TSH]). These patients require no treatment unless they present with elevated TSH and low free T_4.

The electroencephalogram (EEG) is usually normal. Histologic examination of the liver reveals some degree of steatosis. There may be lamellar lysosomal inclusions on electron microscopy [20].

GENETICS AND PATHOGENESIS

The biochemical characteristic of this syndrome is the presence of secretory glycoproteins that are deficient in their carbohydrate content. Terminal trisaccharides are characteristically missing. As a result, a number of glycoproteins become abnormal, including transport proteins, enzymes, hormones such as prolactin and FSH, and coagulation factors. In the initial series of patients, serum activity of arylsulfatase A was recognized to be elevated [1]. Because of the abnormal TBG, it would seem that patients should be detected in programs of newborn screening for hypothyroidism, but that has not been widely encountered.

Among the most used tests for the diagnosis of this condition is the isoelectric focusing of serum transferrin, which reveals the characteristic CDG type I pattern due to the loss of complete N-glycan chains that lead to an increased amount of di- and asialotransferrin at the expense of tetrasialotransferrin [21, 22]. Half of this glycoprotein is found to lack two or four of its terminal sialic acid moieties. The normal transferrin of serum is predominantly tetrasialotransferrin, and there are small amounts of mono-, di-, tri-, penta-, and hexa-sialotransferrins; in the disease state, loss of negatively charged sialic acid causes a cathodal shift. Abnormal transferrin is also present in liver and CSF. Qualitative diagnosis is made by isoelectric focusing and immunofixation of transferrin. Quantitative determination of carbohydrate-deficient transferrin indicated an approximately ten-fold elevation of cathodal transferrin forms [21]. Electrophoresis reveals low molecular weight isoforms of many serum glycoproteins, including α-1 antitrypsin [22, 23]. The diagnostic accuracy may be improved using isoelectric focusing of α-1 antitrypsin and α-1 antichymotrypsin [23], and methodologies such as high-performance liquid chromatography (HPLC) and capillary zone electrophoresis [24] may be better suited to automation. The feasibility of tandem mass spectrometry has been demonstrated to elucidate the glycosylation of transferrin [25], an approach which allows for quantitative results and which offers the specificity to detect variant forms with more subtle differences in glycan processing. In contrast to fibroblasts from healthy controls, which produce mainly $Glc_3Man_9GlcNAc_2$ oligosaccharides linked to the dolichol carrier, fibroblasts of PMM2-CDG patients show an accumulation of shortened dolichol-linked oligosaccharides such as $Man_3GlcNAc_2$ and $Man_5GlcNAc_2$. These represent unsuitable substrates for the oligosaccharyltransferase complex, thereby leading to the loss of oligosaccharide side chains linked to glycoproteins [26].The fundamental defect is in the synthesis and transfer of nascent dolichol-linked oligosaccharide precursors, and incorporation of labeled mannose into glycoproteins and the dolichol-linked oligosaccharide precursor is also shown to be deficient [27]. The abnormality in lipid-linked oligosaccharide biosynthesis could lead to failure to glycosylate sites on proteins and to abnormalities in glycoprotein processing or

function. Furthermore, there is probably a cellular response to unfolded proteins that plays a part in the pathogenesis in this class of disease [28].

Newborn screening for this disease has not been successfully implemented. While it was reported that the transferrin abnormality may be detectable in dried blood spotted on paper, it was not evident in a 19-week fetus [22]. As investigations on the glycosylation state of serum transferrin may prenatally fail [29] or lead to false-positive results in the first three months after birth [30], it has been recommended that testing of transferrin glycosylation should not be performed before three months of age to avoid false-negative results. Mutation analysis can be performed for prenatal diagnostics of PMM2-CDG. Of course, the disease is autosomal recessive, and although some heterozygotes may be recognizable chemically, heterozygote detection is not reliable.

Another pitfall in transferrin diagnostics may be caused by genetic variants affecting charged amino acids that lead to shifts in isoelectric focusing resembling CDG. In these cases, misleading results can be avoided by isoelectric focusing after neuraminidase treatment, which removes sialic acid residues from the oligosaccharide moieties of the protein. Abnormal glycosylation patterns have also to be considered in other diseases as chronic alcoholism, classic galactosemia (Chapter 57), or fructose intolerance.

The defect in phosphomannomutase [4, 5] (see Figure 101.1) can be directly assayed in fibroblasts or leukocytes. In 16 patients, leukocyte activity ranged from 0.02 to 0.08 mU/mg protein as compared with the control range of 1.6 to 2.3. In fibroblasts, the range was 0.1 to 1.4 in patients and 2.2 to 6.4 in controls. In some cases, PMM2-CDG patients, even with severe clinical phenotype, presented with only intermediately reduced phosphomannomutase activities. In any case of suspected PMM2-CDG with an abnormal CDG-I transferrin pattern and only moderately reduced phosphomannomutase activity, genetic analysis of the *PMM2* gene should be performed. The gene for phosphomannose-2 [26], designated PMM2, is localized on chromosome 16p13.3–p13.2, spanning 51.5 kb in eight exons and coding for 246 amino acids. At this point, more than 100 mutations have been described, mostly missense mutations. The disease is pan-ethnic, but different populations have their own set of mutations [31]. The most common mutations are R141H and F119L, accounting for approximately 37 and 17 percent of alleles, respectively; the R141H mutation is found in the compound heterozygous state in approximately 40 percent of patients of Caucasian origin [32], and the combination R141H/F119L accounts for about 38 percent of Caucasian patients. The R141H mutation has never been found in a homozygous state, presumably because that condition is incompatible with life. Patients with the R141H/F119L genotype represent the more severe end of the clinical spectrum. Pathogenic variants at the C-terminal, including p.His218Leu, p.Thr237Met, and p.Cys241Ser, may be associated with a milder phenotype [31, 33]. The F119L mutation has a clear founder effect in the Scandanavian population, and the R141H mutation is associated with a specific haplotype which points to a single ancient mutational event. The observed frequency of the R141H allele (one in 72) in normal populations of Netherlands and Denmark, and the observed frequency of that allele in the compound heterozygous state with other mutations, suggests the frequency of the disease in that population would be expected to be around 1 in 20,000. The incidence in that population, however, has been estimated to be more in the order of 1 in 80,000 [33, 34].

TREATMENT

Treatment is symptomatic. Psychosocial support and genetic counseling for prenatal diagnosis are important to help families cope with the disease and enable informed decisions. Nasogastric feeding and the use of high-caloric diets are helpful in infancy, and painstaking approaches to feeding are required through childhood. Physiotherapy, speech therapy, as well as orthopedic equipment are indispensable. Coagulation abnormalities require special awareness. In case patients develop recurrent stroke(s), small doses of acetylsalicylic acid should be given. Elevated temperature and/or dehydration must be avoided and promptly treated, e.g. with antipyretics and intravenous fluids during intercurrent illnesses but also during anesthesia and surgery. In these situations, electrolytes, blood glucose, coagulation factors (especially factor IX and factor XI) and the proteins C, S, antithrombin III and heparin cofactor II should be closely monitored even if partial thromboplastin time is normal.

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102

Ethylmalonic encephalopathy

MAJOR PHENOTYPIC EXPRESSION

Progressive neurodegenerative disease, acrocyanosis, petechial skin lesions, episodic acidosis, neuroimaging and neuropathologic evidence of basal ganglia lesions, lactic academia, ethylmalonic aciduria, and mutations in the *ETH1* gene.

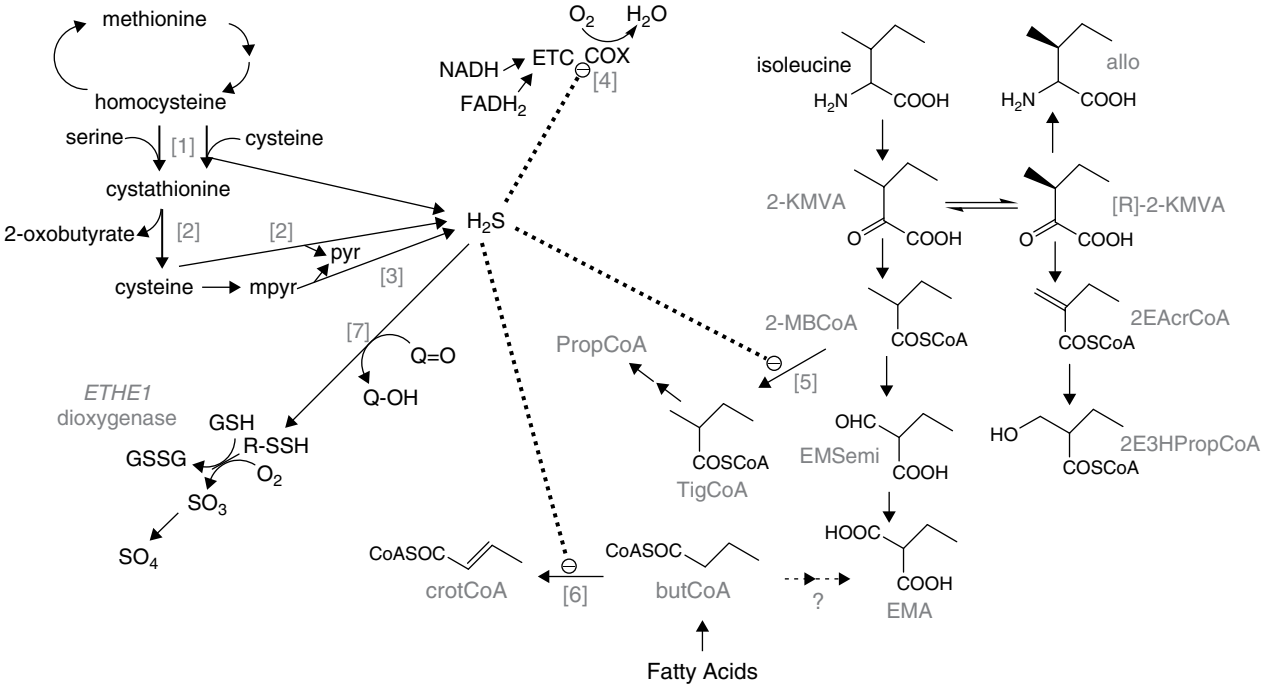

Figure 102.1 Metabolic pathways involved in ethylmalonic encephalopathy. 2EAcrCoA, 2-ethyl-acrylyl-CoA; 2E3HPropCoA, 2-ethyl-3-hydroxy-propionyl-CoA; 2-KMVA, 2-ketomethylvalerate; 2-MBCoA, 2-methylbutyryl-CoA; allo, alloisoleucine; butCoA, butyryl-CoA; COX, cytochrome oxidase; crot-CoA, crotonyl-CoA; EMA, ethylmalonic acid; EMSemi, ethylmalonic semialdehyde; ETC, electron transport chain; PropCoA: propionyl-CoA, TigCoA: tigyly-CoA, Enzymes are marked: (1) cystathionine β-synthase, (2) cystathionine γ-lyase, (3) mercaptopyruvate transulfurase, (4) cytochrome oxidase, (5) methylbutyryl-CoA dehydrogenase/short branched chain acyl-CoA dehydrogenase, (6) short chain acyl-CoA dehydrogenase (7).

INTRODUCTION

Ethylmalonic aciduria (Figure 102.1) is most commonly encountered in what has been termed ethylmalonic-adipic aciduria in less severe forms of glutaric aciduria type II, or multiple acylCoA dehydrogenase deficiency, which is due to deficiency in electron transport flavoprotein (ETF) or ETF dehydrogenase (Chapter 45) [1–3]. It is also encountered in short-chain acylCoA dehydrogenase (SCAD) deficiency (Figure 102.1) [4]; defects in the mitochondrial beta-oxidation pathway leading to ethylmalonic aciduria, but only variable symptoms have occurred in a few patients. Some of these patients present with hypoketotic hypoglycemia, myopathic weakness, or cardiomyopathy, characteristics of disorders of fatty acid oxidation.

A different type of disorder in which ethylmalonic aciduria is associated with a very different phenotype and normal oxidation of fatty acids was first reported in 1991 and 1994 [5–7]. It is recognized most readily by the association of encephalopathy, acrocyanosis, and petechiae. Death in infancy is also characteristic. After the delineation of the molecular defect in the *ETHE1* gene, this disease was distinguished as ethylmalonic encephalopathy [8].

CLINICAL ABNORMALITIES

The most important abnormalities are those involving the central nervous system (Figures 102.2–102.7).

Hypotonia, head lag, and delayed development have been noted as early as three to four months [5–7, 9]. Developmental milestones have failed to be achieved. Generalized tonic-clonic seizures or infantile spasms begin in infancy and may be frequent, and there may be episodes of status epilepticus. Deep tendon reflexes are exaggerated, and there may be ankle clonus and positive Babinski responses. One patient had microcephaly and quadriparesis (Figure 102.4) [7]. Neurologic deterioration is progressive and may be rapid following intercurrent illness and leads to terminal coma and death, generally in the first to fourth year [5, 7, 9].

Manifestations of vascular abnormality (Figures 102.2, 102.3, 102.5–102.9, 102.11, 102.12) are typical in this

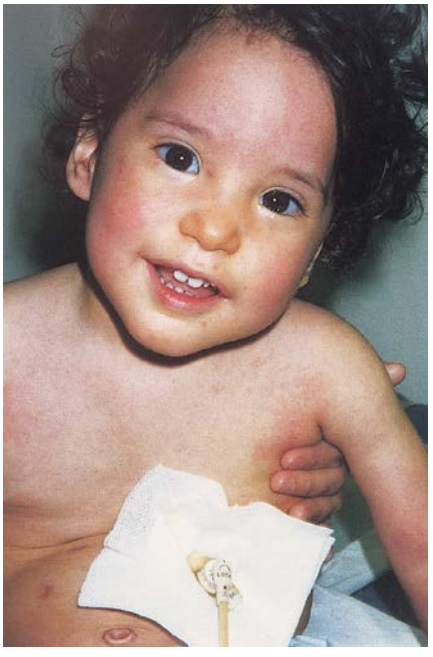

Figure 102.2 SP: A 19-month-old Hispanic-American girl with ethylmalonic aciduria. She was hypotonic and had delayed development. Petechial clusters visible in the forehead, cheeks, and chest came and went.

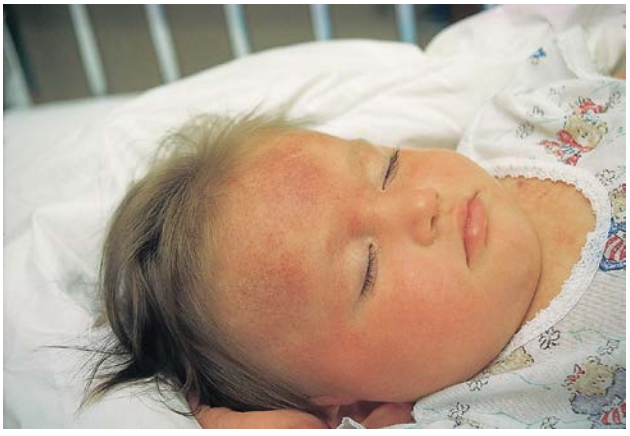

Figure 102.3 AP: A five-month-old girl with ethylmalonic aciduria. The facial appearance and the petechial lesions are illustrated. She was floppy, unresponsive, and had virtually constant infantile spasms. She had epicanthal folds, upslanting palpebral fissures, an upturned nose, and depressed nasal bridge.

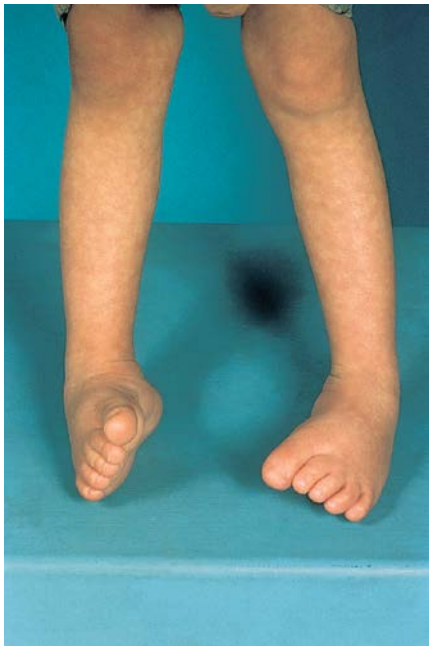

Figure 102.4 FE: A three-year-old Egyptian boy had spastic quadriplegia.

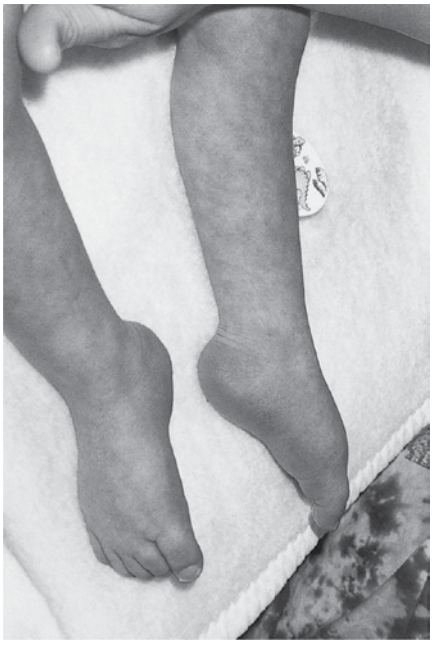

Figure 102.5 SP: The feet and lower legs were cold and alternately pale red or blue.

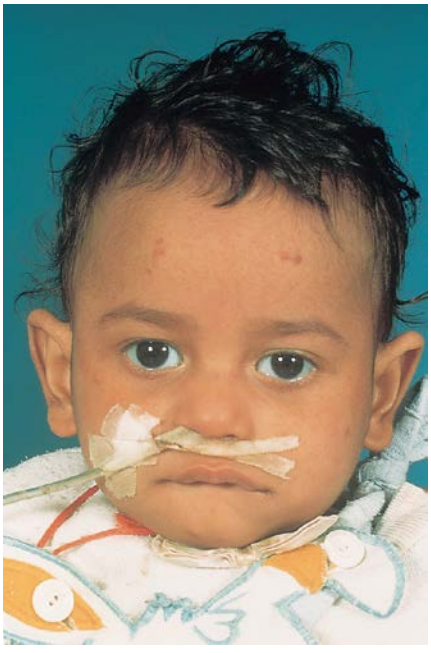

Figure 102.6 MM: A 12-month-old Yemeni boy with ethylmalonic aciduria. There were hemorrhagic spots on the forehead. He also had epicanthal folds.

disease [5–8], and these features are quite unique among metabolic disorders. Acrocyanosis (Figures 102.5) may be the mildest manifestation and it may be associated with edema of the extremities. Patients also have episodic showers of petechiae, often associated with infection. One of our patients (Figure 102.2) was originally investigated for meningococcemia before referral to us. There may also

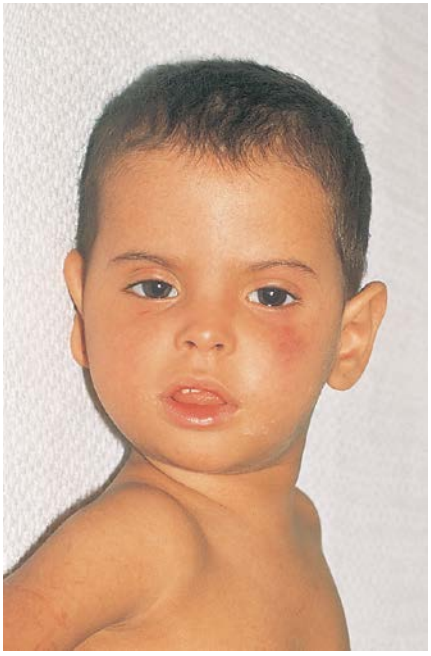

Figure 102.7 TM: A 23-month-old Yemeni girl with ethylmalonic aciduria and a large ecchymotic area on the cheek.

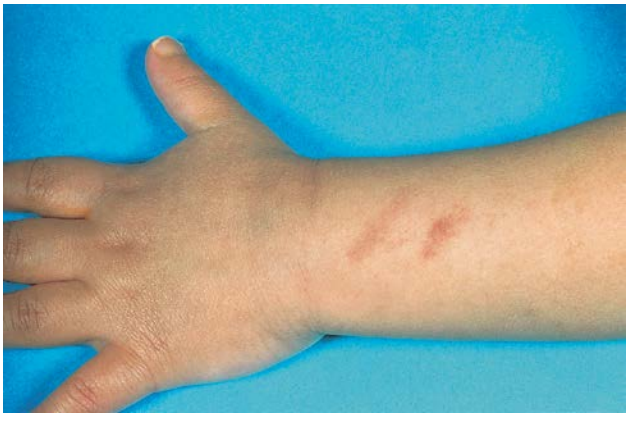

Figure 102.8 FE: At 12 months of age, this Egyptian boy had fresh hemorrhagic streaks on his arm.

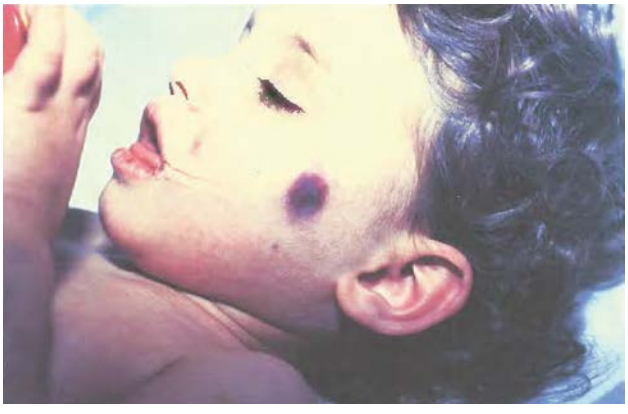

Figure 102.9 MF: A girl with ethylmalonic encephalopathy had a large ecchymotic lesion on her cheek.

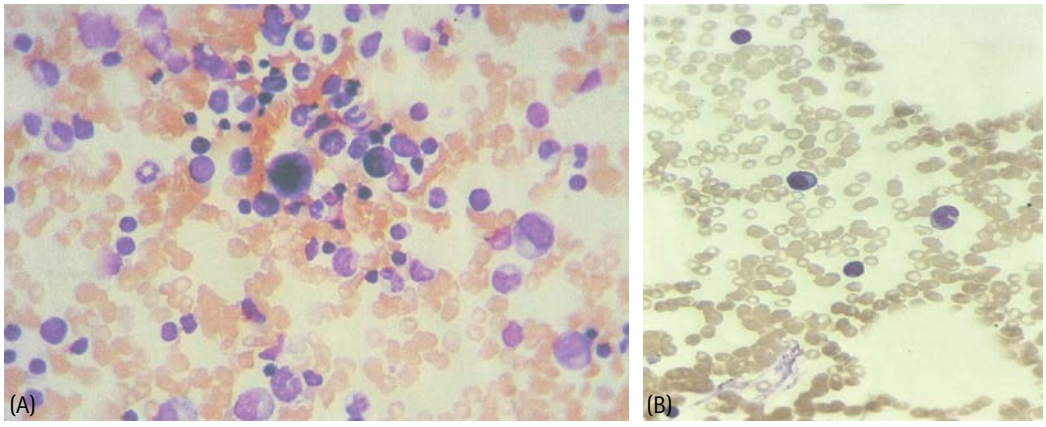

Figure 102.10 Bone marrow aspirates of the patient in Figure 102.9 at 2 (A) and 24 (B) months of age respectively. There was increasing impairment of hematopoiesis involving all three lineages. At two months, there was diminution of mature megakaryocytes despite an increased number of young megakaryocytes. At 24 months, there were only plasma cells, monocytes, and lymphocytes. In parallel, the concentration of hemoglobin F rose to 27 percent.

be ecchymoses (Figures 102.7 and 102.9) or hemorrhagic streaks (Figures 102.8). Ethylmalonic encephalopathy may masquerade as a hematologic disorder and our index patient (Figure 102.9) suffered from progressive pancytopenia (Figure 102.10), in addition to progressive psychomotor retardation. She presented at birth with severe thrombocytopenia unresponsive to cortisone or immunoglobulins [5]. From the second year of life, the patient needed an increasing number of transfusions of platelets and red blood cells. She gradually developed hypersensitivity against HLA identical thrombocytes and finally died in a cardiovascular arrest secondary to severe anemia. In another patient, leukocytosis and thrombocytosis were prominent [10].

Dilated tortuous retinal vessels (Figures 102.11 and 102.12) may be seen as early as three to four months of life. Hematuria may be observed and erythrocytes were reported in the cerebrospinal fluid (CSF) [5, 7, 11]. An association with nephrotic syndrome has been previously reported [12], and we have encountered a case with an episode of nephrosis which was responsive to conventional steroid treatment. One patient had a terminal hemoperitoneum [7]. Biopsies of the skin lesions showed nothing but hemorrhage [7]. There was no evidence for an immunologic abnormality, nor were there abnormalities of bleeding, clotting, or platelets. A markedly elevated level of plasminogen activator inhibitor-1 has been encountered [9]. Terminal events in two patients appeared to be pulmonary edema and one had cerebral edema.

Facial features may be mildly dysmorphic (Figures 102.6, 102.7, 102.13, and 102.14) [5, 7, 9 11, 13]. The facies of these patients tended to resemble each other. Some had epicanthal folds. In most, the nasal bridge was broad and depressed.

Neuroradiologic findings (Figures 102.15 and 102.16) have included frontotemporal atrophy and delayed myelination. In addition, there were areas of high T_2 intensity in the

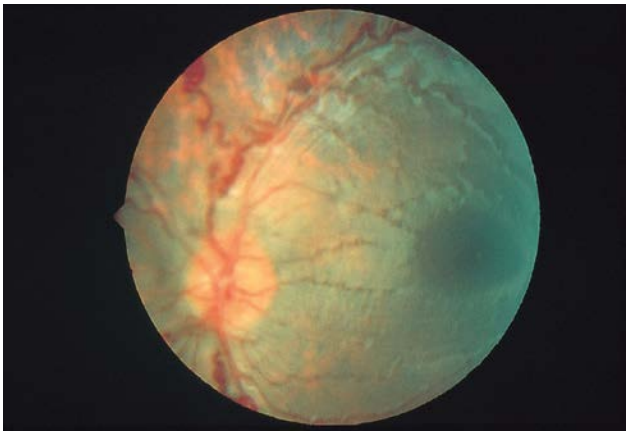

Figure 102.11 Dilated tortuous vessels in the ocular fundus of the patient in Figure 102.8.

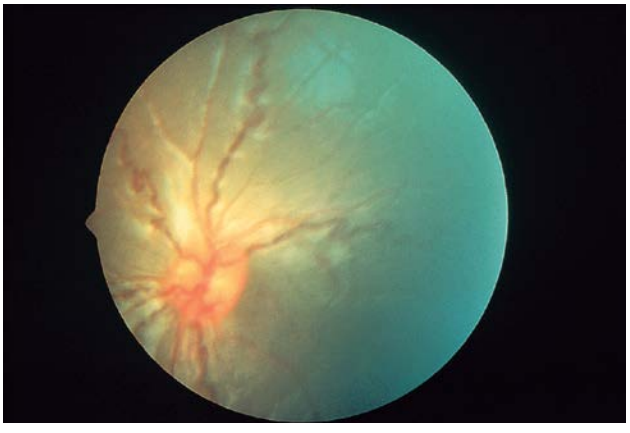

Figure 102.12 Dilated tortuous vessels in the ocular fundus of the patient in Figure 102.4.

Figure 102.13 FE: At 12 months had epicanthal folds and upward-slanting palpebral fissures.

heads of the caudate nuclei, putamina, and posterior fossa [7, 9, 11, 13–15]. Other abnormalities on magnetic resonance imaging (MRI) included cerebral ectopia and a tethered cord [15]. More general findings include atrophy enlargement of subarachnoid spaces [13, 14], and occasionally hyperdensities on MRI T_2 signal of basal ganglia [13]. Electroencephalograms (EEG) were abnormal, revealing multiple focal discharges

Figure 102.14 NS: The nasal bridge was broad and she had epicanthal folds.

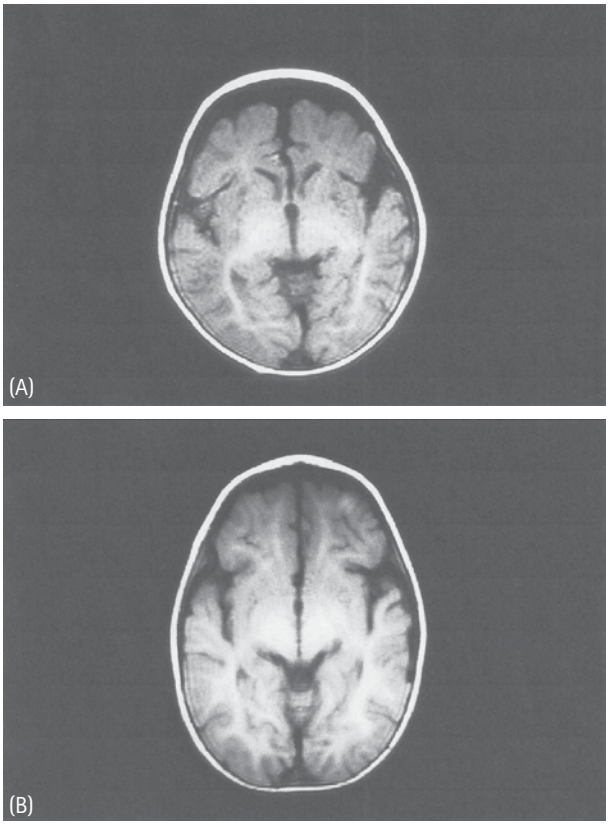

Figure 102.15 Computed tomographic scan of the brain of NS showed frontotemporal atrophy.

or hypsarrhythmia. Studies of nerve conduction indicated sensory neuropathy in the legs [9].

Acute metabolic crises are seen, with lactic acidemia and mild hypoglycemia. Between attacks, the blood concentrations of lactate and pyruvate remained high, and metabolic acidosis is compensated. Concentrations of 3.9–6.0 mmol/L of lactate and 158–230 μmol/L of pyruvate has been recorded; while in the acute attack, levels of lactate as high as 17 mmol/L were found, and there was severe acidosis with pH values of 7.05 and 7.10, and base excess of −19 without ketosis. Liver function tests may be elevated. Creatine phosphokinase (CPK) may be normal or elevated as high as 2100 IU/L [9]. Blood ammonia is normal.

Muscle biopsy revealed increased droplets of lipid, but no ragged red fibers. On electron microscopy, there were mildly increased numbers of pleomorphic subsarcolemmal mitochondria [9 and G. Hoffmann personal observation]. Neuropathologic examination revealed marked capillary proliferation in the substantia nigra, periaqueductal area, putamen, caudate, and medial thalamus (Figure 102.17). Endothelial cells were increased in number and size. There was a relative sparing of neurons and pallor of the background parenchyma that was quite prominent in the substantia nigra. There was vascuolization of white matter tracts.

Patients can have very atypical presentations or comorbidities. In one child with ethylmalonic encephalopathy due to a homozygous mutation in the

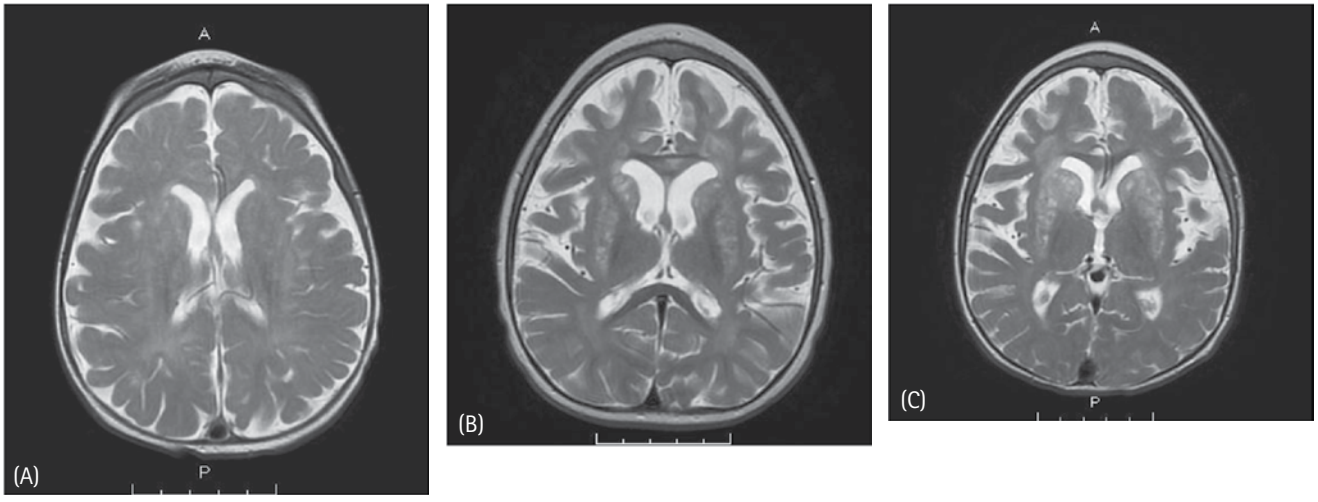

Figure 102.16 Magnetic resonance images (T2 signal) of JG: (A) at six months of age, (B) at three years of age, recently following an influenza A infection, (C) at three years and two months, after two months of treatment with metronidazole and N-acetylcysteine. Note progressive atrophy and encephalomalacia of putamen, caudate, lentiform nuclei, and periventricular white matter with cystic changes. The brainstem and cerebellum appeared normal.

ETHE1 gene (R163W), no pathologic excretion of ethylmalonic acid was found, and the clinical picture was suggestive of a connective tissue disorder (vascular fragility, joint hyperlaxity, delayed motor development, and normal cognitive development) [16]. This patient suggests that ethylmalonic aciduria is not a constant biochemical marker of this disease and that normal excretion of this metabolite, at least between metabolic decompensations, does not exclude this metabolic disorder. Taken together, the clinical course of ethylmalonic encephalopathy is characterized by clinical heterogeneity; Pigeon and colleagues reported different clinical courses even in monochorionic twins [17].

Since the initial report, only about 40 cases of ethylmalonic encephalopathy have been described worldwide, suggesting that this condition is an ultra-rare autosomal recessive disorder. Most patients with ethylmalonic encephalopathy have been, with a few exceptions, of Mediterranean [5, 6, 8, 14] or Arabic [7] descent. However, the actual incidence of this condition could have been significantly underestimated because the biochemical phenotype may be incorrectly attributed to other metabolic disorders, particularly defects of the mitochondrial electron-transfer pathway. Several patients of ethylmalonic encephalopathy were initially diagnosed as glutaric aciduria type II, but this diagnosis was not confirmed by *in vitro* enzyme assays or molecular studies. Some of these patients were proven and more could have been ethylmalonic encephalopathy.

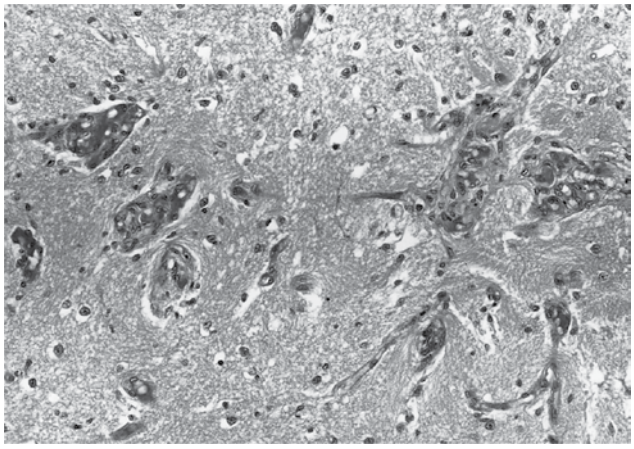

Figure 102.17 Histologic appearance of the caudate nucleus, of NP, the brother of the patient shown in Figure 102.3. He died at eight months. There was marked endothelial proliferation of capillaries and an increase in the number of capillaries (H&E, ×500). (Reprinted with permission from the *Archives of Neurology* [9].)

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion. Boys and girls have been observed in the same family. In two reports [5, 7], the parents were consanguineous. Patients reported have been Yemeni, Italian, Egyptian, Native American, and Hispanic-American.

The major metabolic abnormality is the excretion of ethylmalonic acid in the urine. Amounts reported have ranged from 54 to 2270 mmol/mol creatinine (normal <17). In some patients, the excretion of methylsuccinic acid was also elevated, but in others it was not, and levels were as high as 266 mmol/mol creatinine (normal <12) in one patient. Adipic aciduria was not present, although in one patient a level as high as 334 mmol/mol creatinine was recorded. Tandem mass spectrometry of the blood and urine showed an elevation in C4 and C5 carnitine esters and excretion increased after treatment with carnitine. Levels of free carnitine in blood and urine are low. Acylglycine excretions have been increased, including butyrylglycine and isobutyrylglycine [6, 13], as well as 2-methylbutyrylglycine and isovalerylglycine [13].

In searching for the pathway leading to accumulation of ethylmalonic acid, elevated excretion of this compound and methylsuccinic acid after a load of isoleucine was reported by Nowaczyk *et al.* [18], and an increase in alloisoleucine was also reported. However, in our patient, loading with isoleucine did not change the excretion of ethylmalonic acid or the level of alloisoleucine [9]. Nowaczyk *et al.* [18] postulated a block at the levels of 2-methylbutyrylCoA dehydrogenase; however, the activity of this enzyme was studied by Burlina *et al.* [6] and found to be normal. Furthermore, Ozand and colleagues [7] found the oxidation of ^{14}C-isoleucine to $^{14}CO_2$ to be normal in fibroblasts derived from typical patients. In these studies, the oxidation of ^{14}C-butyrate was normal, consistent with our findings on triglyceride loading. Normal oxidation of fatty acids was also observed by Burlina *et al.* [6].

It is not clear why results were different following isoleucine administration in our patient and the patient of Nowaczyk *et al.* [18]. In our patient, loading with methionine was followed by an increase in excretion of ethylmalonic acid of 1.7 times to 648 mmol/mol creatinine, and that of methylsuccinic acid also rose. A relationship between this syndrome and the metabolism of sulfur amino acids was suggested by Duran and colleagues [19] who found increased excretion of inorganic thiosulfite and an absence of detectable sulfite. They also reported two sulfur-containing acidic amino acids, S-sulfocysteine and S-sulfothiocysteine, each of which can be formed nonenzymatically from thiosulfate and cysteine. The increase in excretion of ethylmalonic acid in our patient following methionine is consistent with these observations.

Methionine is converted normally to homoserine and cysteine. Homoserine is converted to 2-oxobutyric acid which could be a source of ethylmalonic acid. Cysteine is converted to 2-mercaptopyruvic acid which is metabolized to pyruvic acid and thiosulfate and ultimately sulfate [20]. Ethylmalonic acid can be formed via carboxylation of butyryl CoA (Figure 102.1) catalyzed by propionyl CoA carboxylase [21], and this appears to be the source of ethylmalonic acid found in SCAD deficiency and in multiple acylCoA dehydrogenase deficiency [21]. In our patient, loading with medium-chain triglyceride did not greatly increase the excretion of ethylmalonic acid. Ethylmalonic acid could be a product of isoleucine metabolism through the R pathway after racemization of 2-oxo-3-methylvaleric acid, the precursor of alloisoleucine, from the S to the R form, which is then convertible to 2-methylbutyryl CoA, 2-ethyl-3-hydroxypropionyl CoA, and ethylmalonic semialdehyde and then to ethylmalonic acid.

It has been shown that methylsuccinic acid is formed from ethylmalonic acid in bacteria [22]. Methylsuccinic acid may also be made from 4-hydroxyisovaleric acid, which would account for its presence in glutaric aciduria type II, where isovaleryl CoA dehydrogenase is impaired. In fact, the involvement of SCAD (and short-branched chain acyl-CoA dehydrogenase) had been suspected, but conventional enzyme assays have been reported to be normal [13]. The possibility that this disease is a disorder of mitochondrial electron transport had been raised, but studies of mitochondrial DNA in blood and muscle and the enzymes of the electron transport chain (ETC) in muscle and fibroblasts have been reported to be normal [6, 7, 9, 13]. It turns out that the effects on these dehydrogenases and upon the ETC are secondary.

The gene for this disease was found via homozygosity mapping to reside in chromosome 9q13 near the midpoint of the long arm [8]. Physical and functional genomic data and mutational analysis permitted identification of the gene, which has been named *ETHE1*. The protein product is targeted to the mitochondrion and actively translocated [8], where it is cleared of a 7-amino acid leader sequence [23] and internalized into the matrix. A constitutive knockout mouse model was constructed, which demonstrated impaired growth, reduced motor activity, and early death. Deficiency of cytochrome oxidase (COX) was demonstrated in several tissues, with normal activities of other ETC enzymes, including complex I and II [23]. Thiosulfate concentrations were found to be several-fold higher than control, and sulfite was undetectable. Systemic super-physiologic concentrations of hydrogen sulfide (H_2S) were measured and shown to be sufficient to account for the observed inhibition of COX and SCAD [23]. Exposure to air removes the H_2S and relieves the inhibitory effect, which presumably explains previous reports of normal SCAD and COX activities in conventional assays.

Hydrogen sulfide is a volatile molecule which has important toxic effects, but may also serve in some intracellular regulatory functions [24]. The major portion of bodily H_2S arises from bacterial metabolism, but there may also be appreciable endogenous production. Cystathionine β-synthase (using cysteine as an alternate substrate) may produce H_2S in the brain where it acts as a neuromodulator, enhancing the response of N-methyl-D-aspartate (NMDA) receptors [25]. H_2S is also produced in vascular endothelium through the actions of 3-mercaptopyruvate sulfurtransferase and cysteine aminotransferase [26], where it may act as a smooth muscle relaxant [27]. Since the crystal structure of ETHE1 has been identified by Pettinati and colleagues [28], further functional and mechanistic studies on ETHE1 can be done in the future.

Over 30 mutations in *ETHE1* have been found in patients with ethylmalonic encephalopathy [8, 27, 29, 30]. Haplotypes for 47 patients have been tabulated [29]. There have been deletions, including heterozygous [31] and homozygous [32] deletions of exon 4. There have been at least four haplotypes in six unrelated cases which involve the mutation c.487C→T (p.R163W), as well as two other missense mutations at R163 [29], indicating a probable mutational hotspot.

TREATMENT

The disease is generally lethal in infancy or early childhood. Treatment with riboflavin, carnitine, glycine, and vitamin E

have been without evident effect. Ascorbic acid and coenzyme Q10 may be used as well, but there is no formal indication of effectiveness. In our patient [9], a diet restricted in methionine led to a decrease in excretion of ethylmalonic and methylsuccinic acids and normalization of concentrations of lactic acid and bicarbonate, but the disease was relentless, and she died at 11 months of age. It is possible that systematic restriction of sulfur intake may be of some use. Upon determining the basis of the disease, Tiranti *et al.* [8] predicted that there would be a rationale to reduce exogenous H_2S production through drugs that reduce the H_2S-producing bacterial population or to reduce intrinsic H_2S production by bone marrow transplantation. Early trials of broad-spectrum enteral antibiotic (metronidazole at around 30 mg/kg per day) and an agent to increase glutathione (N-acetylcysteine at around 100 mg/kg per day) showed marked benefit in terms of the cutaneous manifestations and, in some cases, also in neurologic symptoms [31–33]. In one patient we followed (Figure 102.17), this regimen did markedly improve cutaneous symptoms, but there was continued neurologic deterioration, central apnea, and ultimately death.

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103

Disorders of creatine synthesis or transport

MAJOR PHENOTYPIC EXPRESSION

Impaired speech and cognitive development, hypotonia, dystonia, seizures, autistic behavior, depletion of creatine in the central nervous system, increased blood and urine guanidinoacetate (in guanidinoacetatetransferase [GAMT] deficiency), low blood and urine creatine (in arginine: glycine amidinotransferase [AGAT] and GAMT deficiencies),and increased urine creatine/creatinine excretion (in creatine transporter [CRTR] deficiency).

INTRODUCTION

The disorders of creatine metabolism consist of three abnormalities in the synthesis and transport of creatine. Each disorder is characterized by severely reduced or absent creatine in the central nervous system (CNS) and neurologic manifestations that range from mild developmental delay to severe neurologic disability. Our recognition of this group of disorders was initiated by Stockler and colleagues [1] in 1996 with the report of GAMT deficiency. The second disorder, AGAT deficiency, was reported in 2000 by Bianchi and associates [2], the third disorder, CRTR, by Salomons *et al*. in 2001 [3].

These disorders provide a novel approach to diagnosis in that in each the creatine peak on proton (H) magnetic resonance spectroscopy (MRS) of the brain is markedly reduced or absent. In GAMT deficiency, the guanidinoacetate (GAA) peak is increased in addition.

In the biosynthesis of creatine, the rate-limiting step is the conversion of arginine and glycine to GAA and ornithine, which is catalyzed by AGAT mainly in the kidney (Figure 103.1). GAA is methylated in the liver to creatine by GAMT; S-adenosylmethionine is the methyl donor. Creatine is transported through the blood and taken up into cells against a high concentration gradient by a saturable Na^+- and Cl^--dependent CRTR (Figure 103.1). Creatine is converted nonenzymatically to creatinine, which is excreted in the urine in amounts approximately equal to the glomerular filtration rate [4].

Biochemical elucidation of the diagnosis is initiated by analysis of the concentrations of creatine and GAA. In AGAT deficiency, GAA concentration in plasma is low, and creatine

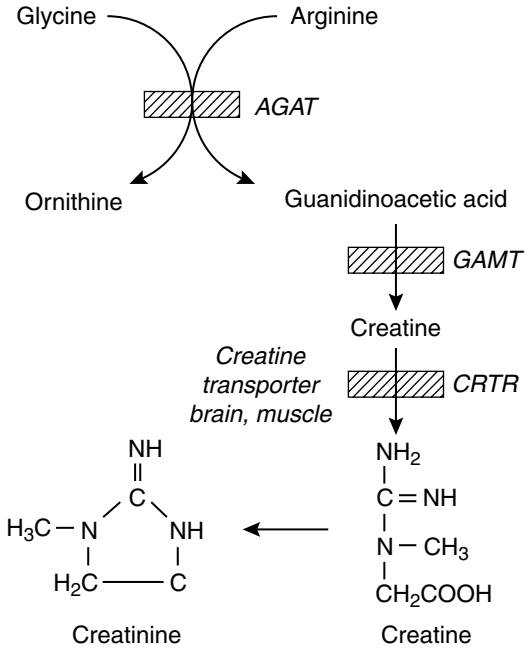

Figure 103.1 Pathways relevant to disorders of cerebral creatine deficiency. Defects are shown in arginine: glycine amidinotransferase, guanidinoacetatetransferase, and the creatine transporter.

excretion in urine is low. In GAMT deficiency, concentrations of creatine are low, and GAA high. In both of these disorders, patients have been recognized by organic acid analysis or amino acid analysis of the urine; since data are reported per unit creatinine excretion. If creatinine is low, most compounds are reported to be high. In CRTR deficiency the excretions of creatine are normal or increased.

Deficiency of enzyme activity has been documented in liver in GAMT deficiency [1] and in fibroblasts in AGAT deficiency [2]. Mutations have been documented in the genes for AGAT deficiency [5] and GAMT deficiency [1, 6]. CRTR deficiency is an X-linked disorder mapped to Xq28. Impaired uptake of creatine by cultured fibroblasts can be demonstrated. The transporter is SLC6A8. A number and variety of mutations have been reported [7].

CLINICAL ABNORMALITIES

The syndromes of cerebral creatine deficiency represent an appreciable cohort of inborn errors of metabolism that interfere with CNS function. An enlarging spectrum of clinical presentation is emerging [7–9], and these diseases are often overlooked. Certainly, patients with CRTR deficiency usually have impaired mental development and seizures. Those with GAMT deficiency may, in addition, have progressive myoclonic epilepsy, but they may have regression with dystonia or hyperkinesis. In contrast, patients with AGAT deficiency, which are much more rare, seldom suffer from seizures but frequently develop progressive muscular weakness at different ages. Most importantly, the syndromes of cerebral creatine deficiency [7–9] should be considered in any patient with developmental delay. The fact that some are effectively treatable [8–11] raises the stakes for early recognition.

Relative frequency is also of interest. In a study of inborn errors of metabolism in patients with unexplained impaired mental development [11], urine samples were studied biochemically in 994 patients, and the frequency of creatine deficiency syndromes was equal to that of phenylketonuria. Similar data have been reported by others [12–14] with CRTR deficiency being most common. A number of studies suggest a frequency as high as 1 percent of patients with unexplained impaired mental development and about 2 percent of males with X-linked mental retardation [15]. These disorders have been considered [16] among the first approach to the algorithmic and chemical investigation of patients presenting with nonspecific impaired mental development.

The index patient with GAMT deficiency presented at 22 months with global developmental delay and severe hypotonia; dystonic movements were noticed from the age of four to six months [1, 10]. By 11 months, he had lost his ability to roll and was unable to sit or crawl. Seizure-like drop attacks were noted over the next few months, and an electroencephalogram (EEG) showed intermittent high-voltage slow runs with some spikes. Magnetic resonance imaging (MRI) at 12 months showed increased signal bilaterally in the globus pallidus and MRS showed absent creatine and increased GAA. GAMT activity in liver homogenate was 1.35 nmol/h. More than 100 patients have been diagnosed with GAMT deficiency since with an estimated incidence in the general population from 1:2,000,000 to 1:500,000 [8, 17].

In addition to intellectual disability, which is present in all affected individuals, epilepsy is the most troublesome manifestation in >85 percent, including myoclonic, generalized tonic-clonic, partial complex, head nodding, and atonic seizures. Seizures are drug resistant in ≈50 percent. Specific therapy may, however, prove successful even in older patients. We have diagnosed a 26-year-old, severely mentally retarded, friendly, unsettled man who had no expressive speech but some comprehension [18]. Head nodding attacks occurred every few seconds to minutes, in addition to atypical absences, and he was wheelchair bound because of frequent astatic episodes. He showed dystonia and involuntary movements as well as an atrophic muscle relief. Three weeks after starting specific therapy of supplementation with creatine and ornithine, as well as arginine restriction seizures had disappeared, and the patient was no longer wheelchair bound (Figure 103.2). One year after starting treatment, he had learned to walk, play games and to draw simple pictures, corresponding to an age of three to four years. Phosphocreatine, which had been severely reduced in muscle, normalized, and he gained about 7 kg in weight.

AGAT deficiency was first reported [2] in two Italian sisters aged four and six with mildly impaired mental development and severe delay in expressive speech development. One sibling had one febrile seizure. MRS studies revealed absent creatine in the CNS. Plasma creatine was normal. Plasma GAA was slightly decreased, and urine creatinine excretion was very low. AGAT activity in

Figure 103.2 27-year-old man with GAMT deficiency in whom treatment was first started at the age of 26 years and resulted in an impressive improvement of seizures, mental capacities, and behavior.

cultured fibroblasts was undetectable. A third patient in this consanguineous family presented at two years of age with developmental delay, absent language, mild hypotonia, and autistic behavior with stereotypic movements of the hand. All three patients had microcephaly. AGAT deficiency appears very rare with less than 20 patients diagnosed to date.

The index patient with CRTR deficiency [3] presented with hypotonia, developmental delay, and seizures. Impaired mental development was documented at six years of age. His mother and maternal grandmother both had learning disabilities; the mother's sister was normal, but their brother had impaired mental development. CNS creatine was absent on MRS. Urine and plasma GAA levels were normal, but creatine levels were increased. Further studies of the three female relatives showed increased creatine in the plasma of the mother and aunt and increased urine creatine in all three women; MRS studies of the mother and aunt showed reduced, but detectable, CNS creatine. The presence of more severe symptoms in the male family members suggested X-linked inheritance, which was later confirmed by finding a mutation in the CRTR gene (*SLC6A8*) at Xq28.

In compilations of 101 male patients [7] and female carriers in unrelated families [15, 19], the most frequent clinical findings in affected males were moderate to severe mental impairment (>80%), expressive speech delay (100%), and behavior disorders (85%; attention deficit and/or hyperactivity, autistic features, aggressive, impulsive, obsessive-compulsive, self-injurious and/or stereotypic behaviors). Seizures were common (60%), mostly responsive to antiepileptic therapy. Other less frequently reported symptoms included hypotonia (40%), spasticity (26%), movement disorders (≈30%: wide-based gait, ataxia and/or dystonia or athetosis), midface hypoplasia, short stature, and gross motor delay. Head circumference was variable. Feeding difficulties, frequent vomiting, failure to thrive, and other gastrointestinal issues are additional clinical features of the disease. Adult patients can develop ptosis, external ophthalmoplegia, or parkinsonism as well as chronic constipation leading to megacolon, ileus, or bowel perforation. However, life expectancy appears to be normal. Female carrier patients can have variable degrees of learning disability and can present as the index patient with mild-moderate disability, behavioral problems, and seizures. *SLC6A8* deficiency was found with a prevalence of 2 percent in 408 patients with nonsyndromic X-linked impaired mental development [15]. MRS studies showed almost complete absence of creatine (Figure 103.3). An MRI showed abnormal increased signal in the periatrial white matter on the T_2-weighted images, and abnormal signal intensity in bilateral globi pallidi (Figures 103.4–103.6). Most patients have normal brain imaging.

The creatine deficiency syndromes are all characterized chemically by depletion of cerebral creatine, which is demonstrable by MRS. Measurement of GAA in body fluids discriminates between GAMT deficiency in which it is high and AGAT deficiency in which it is low (Table 103.1). In GAMT deficiency plasma, GAA concentrations are 2–200 times elevated in urine and 10–200 times the upper limit of normal in plasma [8]. In CRTR deficiency, they are normal. Creatine concentrations in blood and urine are low in AGAT and GAMT deficiencies, while the urinary creatine to creatinine ratio is elevated in CRTR deficiency. The molar ratio of creatine-to-creatinine in random urine of affected males is greater than 3.0 (normal <1.7 in children). However, false positives can occur and should be followed-up by examining a repeat morning urine sample collected after a one-day meat- and fish-free diet. Hemicygous females cannot be diagnosed reliably by biochemical testing.

Recently, experience has enlarged the clinical spectrum of GAMT deficiency [8], but all have presented with moderate

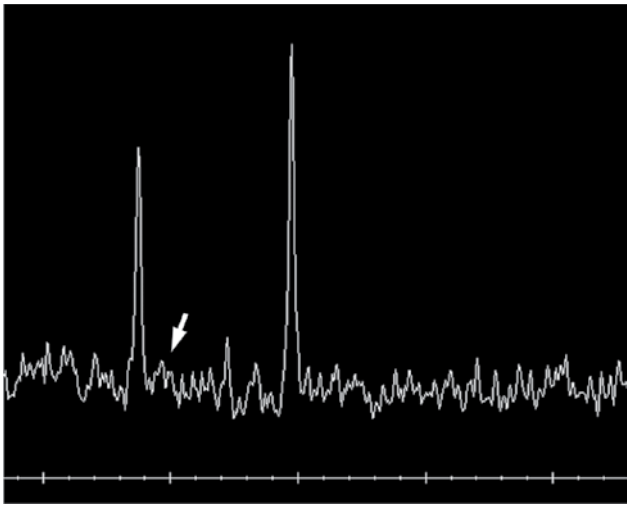

Figure 103.3 MRS of the brain of a patient with creatine transporter deficiency. Single voxel MR spectrum with TE = 144, demonstrating reduction in the creatine peak (arrow).

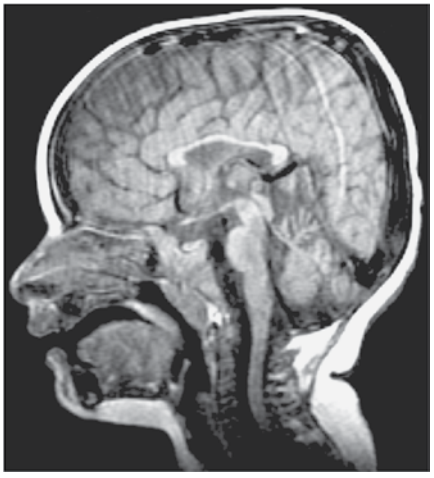

Figure 103.4 MRI of the brain of a patient with creatine transporter deficiency. This sagittal T_1 image shows marked abnormal thinning of the entire corpus callosum.

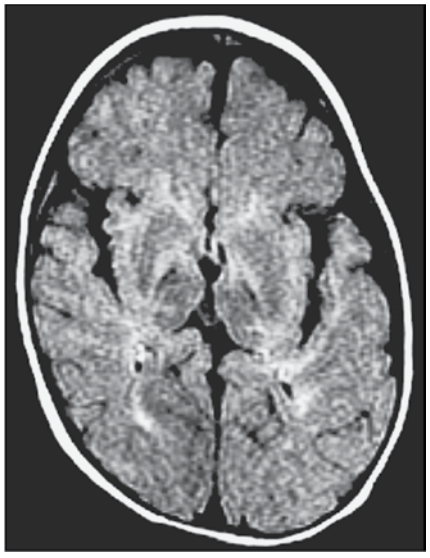

Figure 103.5 Axial FLAIR MRI of the brain illustrating abnormalities in the periventricular white matter and globus pallidus.

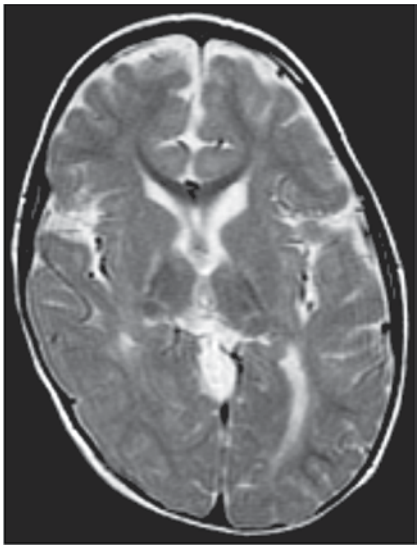

Figure 103.6 MRI of the brain. This T_2-weighted image demonstrates abnormal signal intensity in the periventricular white matter and globus pallidus bilaterally.

to severe delay in development and absent or minimal speech. Regression of skills has been observed, and one patient became wheelchair bound at 14 years of age. Imaging of the brain may be normal, or there may be delayed myelination and hyperintensity of the globi pallidi. Levels of creatine are low in the cerebrospinal fluid, as well as in the plasma and urine.

GENETICS AND PATHOGENESIS

The common biochemical abnormality depletion of creatine in the CNS is likely to be the primary pathogenic mechanism. Patients with GAMT and CRTR deficiencies have a movement disorder, and many have imaging abnormalities in the basal ganglia. Guanidinacetic acid is a known neurotoxin and a potential epileptogenic agent [1]. However, patients with CRTR deficiency in whom plasma GAA is normal have also presented with status epilepticus.

Creatine and phosphocreatine play an important role in intracellular energy metabolism as a high-energy buffering system through the reversible reaction catalyzed by creatine kinase in which creatine and adenosine triphosphate (ATP) form phosphocreatine and adenosine diphosphate (ADP) in the mitochondria. Recent data also suggest that creatine may act as neuromodulator or true neurotransmitter [20].

Approximately 95 percent of creatine is found in skeletal muscle, with the rest distributed between the CNS, liver, and kidney. Creatine and phosphocreatine are non-enzymatically converted at an almost constant rate (~1.7%/d) into creatinine, which passively diffuses out of the cells and is excreted by the kidneys into the urine. The urinary creatinine excretion therefore represents a convenient indicator of the total creatine stores in the body. Some 50 percent of creatine is synthesized *de novo*, primarily in the liver, kidney, and pancreas; the rest is from dietary sources. Uptake into tissues is accomplished by a specific Na^+/Cl^--dependent CRTR (Figure 103.1). As the brain is also capable of creatine synthesis, and creatine levels in CSF of CRTR deficient patients are normal or even elevated, the pathophysiology of CRTR deficiency seems to derive from defective reuptake after release of creatine in agreement with its role of neuromodulator of neurotransmitter [20], similar to the dopamine transporter deficiency syndrome (Chapter 17).

Table 103.1 Biochemical differentiation of creatine deficiency syndromes

	GAMT Deficiency	AGAT Deficiency	CRTR Deficiency	
			Males	Females
Plasma/urine guanidinoacetate	High	Low	Normal	Normal
Plasma/urine creatinine	Low	Low	Low-normal	Normal
CSF creatine	Low	Low	Normal-high	No data
Urine creatine	Low-normal	Low normal	Normal-high	Normal-high
Urine creatine/creatinine	Low	Low	High	Normal-mildly high

AGAT and GAMT deficiency are inherited as autosomal recessive disorders. Mutation analysis of the index patient with AGAT deficiency and 26 family members revealed the homozygous mutation W149X [5]. If molecular genetic test results are inconclusive (i.e. if sequence variants of unknown significance are identified), GAMT enzyme activity (in cultured fibroblast or lymphoblasts), AGAT enzyme activity (in lymphoblasts), or creatine uptake in cultured fibroblasts can be assessed. Enzyme activity in AGAT deficiency has been found to be undetectable in fibroblasts derived from patients and intermediate enzyme activity for the heterozygotes [2, 5]. In GAMT deficiency, enzyme activity in the index patient was 1.35 nmol/h in biopsied liver [1].

In GAMT deficiency, the gene has been mapped to chromosome 19p13.3; more than 50 mutations were reported [1, 6, 8] comprising nonsense and missense variants, splice errors, insertions, deletions, and frameshifts (for reported mutations, see: http://www.LOVD.nl/GAMT). The majority of pathogenic variants (>60%) are missense. The most frequent mutations in GAMT have been the splice site mutation c.327G>A detected in about 25 percent of pathogenic variants [6, 8], which has shown no ethnic boundaries, and c.59G>C in about 20 percent. The majority of mutations defined have been null mutations.

CRTR deficiency is coded for by a gene on the X chromosome Xq28 [21]. CRTR mutations have included missense, nonsense, and single amino acid deletions [3, 7] (see: http://www.LOVD.nl/SLC6A8). However, large deletions, such as Arg514 to ter have been reported [3]. The frequency of mutations in the *SLC6A8* gene has been compared to that of the fragile X mutation [7, 15]. Patients with mutations in *SLC6A8* have been shown to have defective uptake of creatine in cultured fibroblasts [3, 7, 22]. Four missense mutations showed residual activity of the transporter and appeared to lead to a milder and more variable phenotype [7].

TREATMENT

Treatment with supplemental creatine (CR), a dose of 400 mg/kg per day, was employed and resulted in significant improvement in patients with AGAT and GAMT deficiencies. In three patients with AGAT deficiency, there was rapid improvement in fine-motor skills and in behavior disorder; these improvements paralleled the increase in brain levels of creatine. Speech and cognition remained significantly impaired. In GAMT deficiency, oral supplementation in the first three reported patients, with creatine monohydrate in doses of 0.35–2.0 g/kg per day, resulted in gradual increase in the CNS creatine signal on MRS, but it was still significantly below normal. GAA levels remained high, suggesting the possibility that the compound may inhibit the transport of creatine into the CNS. The index patient did show significant clinical improvement; the movement disorder, swallowing difficulties, and seizure disorder resolved. His gross motor function improved, and he was able to walk at the age of five years. He continued to have some autistic and self-injurious behavior. Another patient showed improved psychomotor function and resolution of the seizures and globus pallidus lesions. A third showed no improvement; seizures continued. In our patients, dietary arginine restriction and ornithine supplementation (100 mg/kg per day) with an arginine-free essential amino acid formula (0.4 g/kg per day), resulted in significant reduction of plasma GAA and improvement in seizure activity and EEG [18, 23]. Current treatment with 400(–800) mg/kg of creatine and ornithine and restriction of arginine intake has led regularly to improvement [8]. Replenishment of the cerebral creatine pool takes months to years and is not complete. Most patients have reached a plateau, but in our patients, therapeutic result has been rewarding. Presymptomatic treatment of neonates has resulted in normal development [24], and we have seen near normal development in patients which were diagnosed and treated since infancy, diagnosed after a first seizure. Early diagnosis and initiation of treatment in the first months of life appears to prevent development of symptoms, as also shown in the brother of two AGAT-deficient sisters, who was diagnosed at birth, treated pre-symptomatically with oral Cr and did not develop the neurologic symptoms of AGAT deficiency [25]. Creatine supplementation therapy should be monitored for the possible development of creatine-associated nephropathy [26]. Inclusion of GAMT deficiency into newborn screening programs has now been developed with measuring GAA in blood spots and GAMT gene sequencing in positive samples [27].

Supplementation with creatinine in doses as high as 750 mg/kg per day and other treatment strategies such as to enhance endogenous creatine synthesis through supplementation with the precursors S-adenosylmethionine, glycine and arginine did not result in improvement in clinical manifestations or MRS creatine signal in patients with CRTR deficiency. Affected females, who have residual Cr transport capacity, may benefit from supplementation with Cr [28].

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GLUT1 deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypoglycorrhachia, seizures, mental retardation, movement disorders, paroxysmal exercise-induced dyskinesia (PED), dystonic tremor of limbs and voice (DT), low CSF lactate, defective activity of the GLUT1 glucose transporter (SLC2A1).

INTRODUCTION

GLUT1 deficiency was first described by De Vivo and colleagues in 1951 [1] with the report of two patients in whom infantile seizures, developmental delay, and acquired microcephaly were associated with low concentrations of glucose in the cerebrospinal fluid (CSF) despite normal concentrations of glucose in the blood. The concentrations of lactate in the CSF were also low. They postulated defective transport of glucose from the blood to the CSF. They showed that transport of glucose into isolated erythrocytes was lower than in control cells.

Wang [2] documented the presence of mutations in the *GLUT1* gene. The 15 patients were heterozygous; therefore, these mutations were dominant. A patient with autosomal recessive mutations in *GLUT1* was reported in 2010 by Rotstein *et al.* [3]. The gene is on chromosome 1at p35-p31.3.

CLINICAL ABNORMALITIES

Quite a wide spectrum of clinical manifestations has been observed. Onset has often been with seizures, in which apneic episodes, staring spells or episodic eye movements have presented in the first four months in the classic, more severe phenotype of dominant disease. The 15 patients reported by Wang [2] had seizures, developmental delay and progressive microcephaly. The recessive patient of Rotstein [3] had a severe clinical picture characterized by stiffening of the limbs and cyanosis at six weeks of age, staring spells, myoclonic jerks and tonic clonic seizures, and delayed psychomotor developmental. At six years IQ was 42. He had axial hypotonia, spastic extremities, and severe ataxia. At the other end of the spectrum was a family with mild to severe seizures, delayed development, and ataxia [4].

A classic phenotype observed in 81 percent of 16 patients [5] was that of infantile seizures, acquired microcephaly and spasticity. Seizure types varied, and were typically unresponsive to anticonvulsant medications. Cognitive defect ranged from severe mental retardation to mild learning disability. Neurologic involvement included variably pyramidal, extrapyramidal, and cerebellar systems.

Seizures were not invariable [6]. Nevertheless, a 6-year-old boy had psychomotor delay, nystagmus, dysarthria, ataxia, and dystonic posturing. Three patients without seizures [5] had mental retardation, dystonia, dysarthria, and ataxia. Another atypical patient had paroxysmal blinking and abnormal movements of the head and eyes [5]. A patient with early-onset ataxia began having seizures at 13 years [7]. Cognitive and motor skills degenerated at this time. Electroencephalogram (EEG) revealed background slowing.

Exercise-induced dystonia was reported in two girls [8]. This picture has been listed as GLUT1 deficiency syndrome 2 (MIM 612126). Patients have had in addition to the dyskinesia, choreoathetosis, absence epilepsy, and mental retardation that is usually mild.

Exercise-induced dystonia was reported as early as 1984 [9]. It included involuntary movements of the legs after walking or of the arms after writing. Most patients with this syndrome have not had seizures, but there were exceptions [10]. In some, there were mild learning disability or irritable impulsive, aggressive behavior. Onset of the dystonia may be as late as 12 or 15 years of age [11]. This syndrome expresses as a dominant and has been observed over three generations [12], and five generations [13]. EEG has been normal in as many as 43 percent, but others have had interictal spike discharges even in patients without seizures [12, 13].

Intrafamilial variation has been reported [14] ranging from asymptomatic carriers of mutation to a broad spectrum of seizures.

Heterozygous mutations in this syndrome were reported by Margari [10] and Munchau [11] in 2000.

Dystonic tremor has been reported [15] in GLUT1 deficiency. The tremor involves the limbs and the voice, and it may be the only manifestations of disease. Generalized action tremor was typified by tremor of the writing hand, which could be controlled by holding it with the left hand. A tremor made carrying a glass of water an adventure. The voice was described as jerky. This patient also developed paroxysmal exercise-induced dystonia. Another patient with tremor and a jerky voice had paroxysmal exertional dystonia. A sitting position could induce a right-foot dystonia and lower limb clonic tremor, while vocal tremor could be accentuated by producing a sustained sound. EEG revealed slow-wave discharges.

Electrophysiological studies of patients with tremors revealed 6–8.5 Hz tremor associated with bursts of 80–170 ms duration [15].

The disease in all of its manifestations is characterized by low concentrations of glucose in the CSF. Reported concentrations have often been not very low, e.g. 2.5 mmol/L (control 2.7–4.9) [15]. The CSF/serum glucose ratio is similarly low, e.g. 0.57 (control >0.6), and the CSF concentration of lactate is low. In some reports, the CSF levels of glucose were normal (2.6–2.8 mmol/L) but the ratios were low, 0.5–0.59 [13, 14, 17].

Imaging of the brain by CT or MRI may be normal [15], but severe cortical-subcortical atrophy has also been observed [16].

Macrocytic hemolytic anemia and reticulocytosis has been reported in a three-generation family [17].

It seems likely that the great variability in clinical manifestations reflect varying degrees of deprivation of glucose to the brain. This could be a function of the degree of change in the transporter, but it could also reflect environmental changes including infection and exercise. In a comparison of a patient with GLUT1 deficiency and a patient with pancreatic nesidioblastosis the unifying pathogenesis pointed out was decrease in the supply of glucose to the brain [18].

GENETICS AND PATHOGENESIS

In a majority of patients, mutations in the *GLUT1* gene expressed as dominants, but recessive homozygotes have been reported.

The gene *SLC2A1* has been mapped to chromosome 1p34.2 [5]. The gene codes for the major transporter of glucose in brain and erythrocytes [19]. The gene was cloned from human HepG2 hepatoma cells [20].

A heterozygous deletion in the gene was found [21] in one of the original patients of De Vivo [1]. In another, a heterozygous c.1526C>A change leading to p.Y449X termination was found in a patient with severe disease [21]; while in another patient, with severe disease c.1545A>T led to a truncated p.K456X [21]. A p.137T>A missense mutation was found in a mother and daughter with dystonic tremor [15].

A de novo heterozygous mutation p.34N>I was reported in a dystonic boy without seizures [6]. In the family with paroxysmal exertion-induced dystonia and hemolytic anemia there was a heterozygous 12bp deletion (1022-1033 del) in a transmembrane segment [17].

Mutational analysis has been reported [15] as an initial diagnostic procedure. This would permit the avoidance of lumbar puncture. Also, it is recognized that the glycorrhachia may not be present in all patients at all times.

Transport of glucose has been studied *in vitro* in erythrocytes of patients and controls by measuring the sodium independent transport of 14C-labeled 3-0-methylglucose [22]. In patients, it was 35 percent of control.

Decrease in the supply of glucose to the developing brain has been classified as infantile neuroglycopenia [18]. The authors attributed to glucose dual functions as fuel and as a signaling molecule.

TREATMENT

A ketogenic diet has been reported to lead to control of seizures and major clinical improvement [23, 24]. In patients treated with a ketogenic diet, concentrations of hydroxybutyrate were inversely correlated with base excess [25]. In a series of four patients, all treated patients remained seizure free [25]. The only adverse effect was the occurrence of renal stones in one patient.

The ketogenic diet did not improve cognitive function [5]. Barbiturates are known inhibitors of glucose transport via GLUT1. In studies of 0-methlglucose transport in erythrocytes [22], barbiturates significantly reduced transport in both patient and control cells. The data argue against the use of barbiturates in the treatment of seizures in this disease. Ethanol, diazepam, and chloralhydrate were also found to inhibit transport [26]. Phenytoin and carbamazepine anticonvulsants were not inhibiting, and therefore were preferred as agents.

Thioctic acid was found to increase transcription of the *GLUT1* gene in fibroblasts derived from patients [27]. 0-methylglucose uptake in fibroblasts was also increased.

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Hypophosphatasia

MAJOR PHENOTYPIC EXPRESSION

Impaired calcification of the matrix of bone and of cartilage. A range of phenotypes has been described from a lethal neonatal or really prenatal form to attenuated forms manifested by loss of teeth, or simply high levels of phosphoethanolamine in asymptomatic individuals.

In the lethal neonatal form, bowing, shortening, and multiple fractures of long bones, cutaneous dimples, and respiratory insufficiency result from abnormalities of the ribs; and bulging fontanel or craniosynostosis.

In childhood forms, bony abnormalities lead to shortness of stature; craniostenosis is a feature, and there is premature loss of deciduous teeth.

Adult-onset forms may have tooth loss only, but bone pains are common in all forms.

All these types have elevated urinary excretion of phosphoethanolamine, and low activity of the tissue nonspecific, liver, bone, kidney isozymes of alkaline phosphatase. The gene is known as *ALPL*.

INTRODUCTION

Hypophosphatasia was first recognized as a distinct clinical entity by Rathbun in 1948 [1], though it is clear that patients with this disorder had been described previously, usually without measurement of the serum activity of alkaline phosphatase [2–6]. The deficient isozyme is referred to as TNSALP [7] (tissue nonspecific alkaline phosphatase) There is a spectrum of severity representing genetically distinct disorders. A number of different phenotypes have been delineated [3]. The most severely affected phenotype is one in which presenting manifestations are at birth. A few patients have been diagnosed *in utero*. The second, or childhood type, is characterized by a more gradual development of disease, moderately severe rachitic changes in the skeleton, and premature loss of teeth. The third phenotype is an adult-onset disease in which there are milder symptoms of bony disease. Some have simply odontohypophosphatasia in which there is loss of deciduous or even permanent teeth because of defective formation of cementum. Individuals, who may represent a distinct disorder, or fourth phenotype, are adults with no symptoms found to have hypophosphatasia, because an alkaline phosphatase assay was included in a routine chemical panel, or assay of amino acids in urine turned up phosphoethanolamine.

The diagnosis is usually made by assay of the enzyme in blood serum, but it is important to employ age-appropriate normal reference ranges. It is possible to assay for the specific bone isozyme [8]. The excretion of phosphoethanolamine in urine can be determined on an automatic amino acid analyzer permitting ready distinction from normal [2] (Figure 105.1). This compound is also elevated in blood. Pyridoxal-5-phosphate (PLP), which like phosphoethanolamine is a substitute for alkaline phosphate, is also elevated in plasma.

The *ALPL* gene is located on chromosome 1, at band 1p36 [9]. A number of predominately missense mutations has been reported [10–14].

CLINICAL ABNORMALITIES

In the most severe prenatal or neonatal form (Figures 105.2–105.7) the cranium is soft and globular, giving the picture of a boneless skull (Figure 105.4). Skull films reveal a well-calcified base and marked lack of calcification of

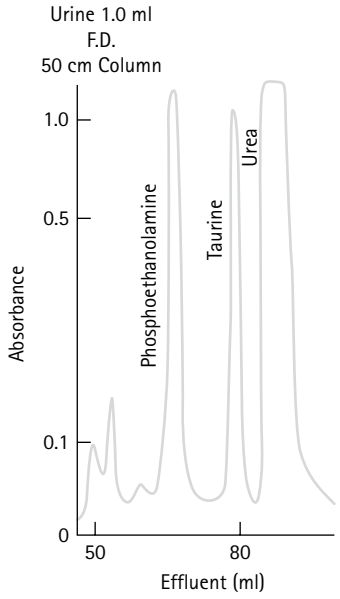

Figure 105.1 Phosphoethanolamine excretion quantified on an early model automatic amino acid analyzer.

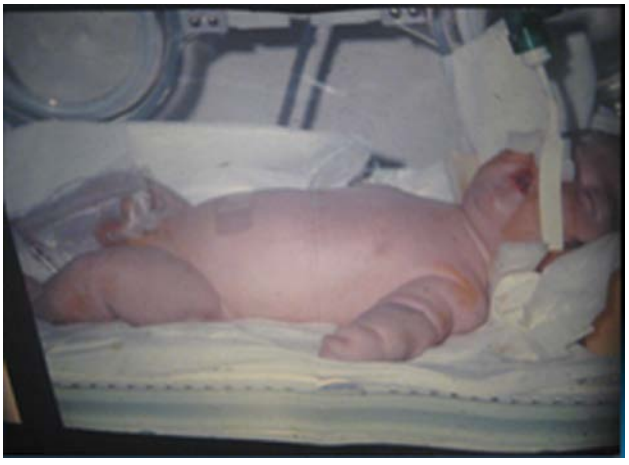

Figure 105.2 Neonatal hypophosphatasia. The baby had short, deformed limbs. The legs were bowed and had prominent dimples of the skin. (Reproduced with permission from Nyhan, WL. and Sakati, NO, *Genetic and Malformation Syndromes in Clinical Medicine*. Year Book Medical Publishers, Inc. Chicago, 1976.)

the other bones. There may be patchy ossification of the frontal bones and small plaques of parietal or occipital bone. Deformities of the extremities are pronounced (Figures 105.2 and 105.3). The extremities are short. Dimples are characteristically present, whether or not there is bowing [15]. Roentgenographic examination [3, 4, 16] reveals generalized rarefaction of the skeleton. The roentgenographic abnormalities of this disease distinguish it from all other disorders of bone. The long bones (Figures 105.6 and 105.7) have irregular and incomplete ossification in the metaphyseal areas, with deep segmental defects. They

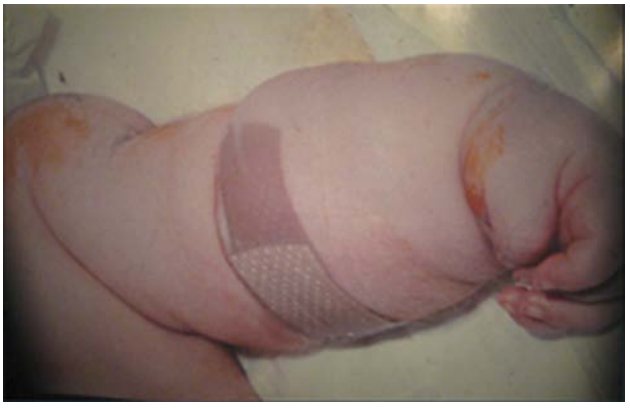

Figure 105.3 The arm, like the other extremities, was short and rather puffy in appearance. Prominent dimples indicated underlying fractures. (Reproduced with permission from Nyhan WL, and Sakati NO, *Genetic and Malformation Syndromes in Clinical Medicine*. Year Book Medical Publishers, Inc. Chicago, 1976.)

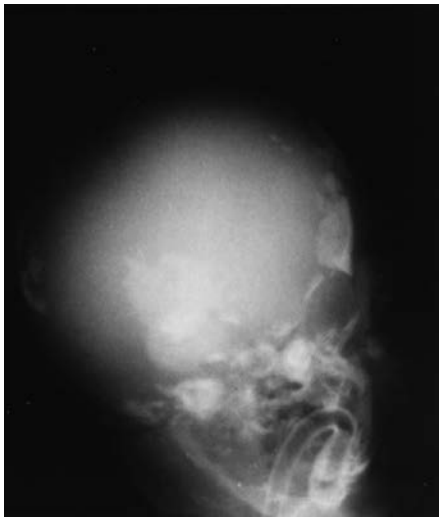

Figure 105.4 Roentgenogram of the skull. There was very poor ossification. Only scattered islands of bone were present. (Reproduced with permission from Nyhan WL, and Sakati NO, *Genetic and Malformation Syndromes in Clinical Medicine*. Year Book Medical Publishers, Inc. Chicago, 1976.)

are also bowed and there are often associated overlying skin dimples. Multiple fractures are the rule. Thoracic cage defects and short ribs (Figure 105.5) lack proper bony support for the thoracic cavity. Pulmonary ventilation is deficient and pneumonia is a common complication.

These patients may be stillborn or die within hours of birth of respiratory difficulty, or shortly later of pulmonary infection. They fail to thrive; they may have vomiting, fever or convulsions. Loud crying, as if in pain, has been observed even in very young infants.

A moderately severe or infantile form may present within the first months of life. There have been no survivals in patients with hypophosphatasia presenting with clinical manifestations prior to the end of the first six months.

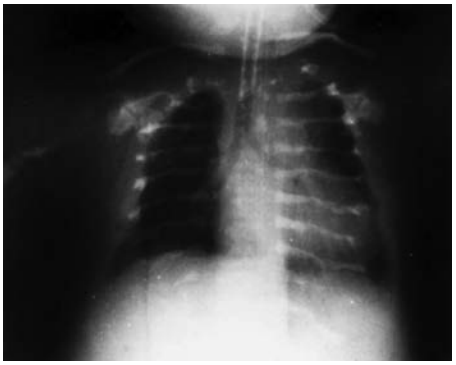

Figure 105.5 Roentgenogram of the chest. The ribs were ribbon-like, delicate, and short. There was poor ossification and multiple fractures. (Reproduced with permission from Nyhan WL, and Sakati NO, *Genetic and Malformation Syndromes in Clinical Medicine*. Year Book Medical Publishers, Inc. Chicago, 1976.)

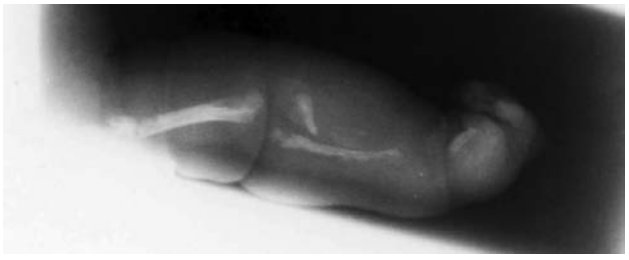

Figure 105.6 Film of the upper extremities revealed incredibly poor ossification and irregularity of the metaphyses. There were multiple fractures of the humerus, ulna, and radius. (Reproduced with permission from Nyhan WL, and Sakati NO, *Genetic and Malformation Syndromes in Clinical Medicine*. Year Book Medical Publishers, Inc. Chicago, 1976.)

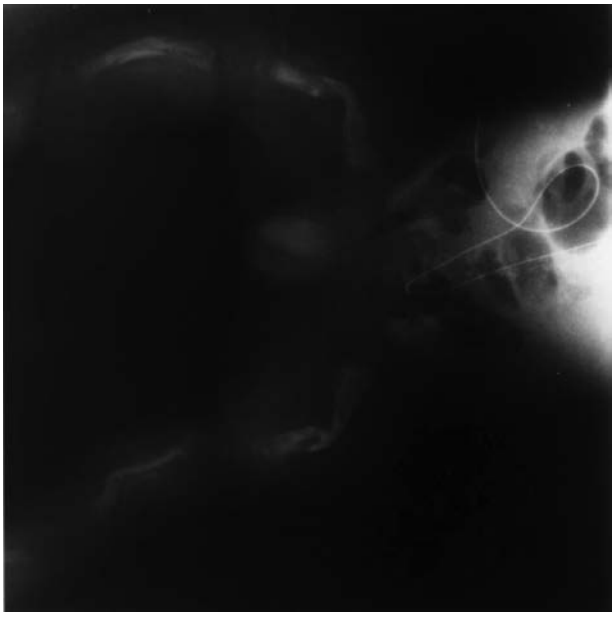

Figure 105.7 Roentgenogram of the lower extremities. The bones were demineralized and ribbon-like. Marked angulation of both femora was interpreted to represent old fractures. The metaphyses were irregular, and ossification was markedly deficient. The tibiae were bowed and showed multiple fractures. (Reproduced with permission from Nyhan WL, and Sakati NO, *Genetic and Malformation Syndromes in Clinical Medicine*. Year Book Medical Publishers, Inc. Chicago, 1976.)

These infants also have generalized skeletal deformities. The cranial sutures are wide, and a bulging anterior fontanel and prominent scalp vein usually develop. A rachitic type of thoracic rosary may be present.

In patients with hypophosphatasia first seen after the seventh month of life and throughout childhood, the disease may be less severe. There are, nevertheless, generalized skeletal abnormalities. It is characterized by premature loss of deciduous teeth [17–19]. Other dental problems include dental hypoplasia and marked dental caries. Craniostenosis is a common sequel in these infants. Premature synostosis occurs in the presence of uncalcified osteoid [20]. Exophthalmos and increased intracranial pressure may ensue. The roentgenogram of the skull may assume a beaten silver appearance [21, 22]. Convulsions, brain damage, or even death may be complications in the absence of surgical decompression. Walking is often delayed and awkward. The lower extremities may be bowed or there may be genu valgum. These patients may have retarded growth and increased susceptibility to infection. Roentgenograms reveal irregular epiphyses and radiolucencies in the bony shafts. There may be features suggestive of rickets with costochondral beading and widening of the ends of the bones, as well as the bowing deformity but, on X-ray, the ends of the bones have a notched appearance very different from the cupping of rickets and more like that of a metaphyseal dysplasia. The occurrence of subperiosteal new bone formation also distinguishes this picture from that of rickets [23]. There is considerable variation in severity among these patients. In fact, some have been observed in whom premature loss of the anterior deciduous teeth was the only evidence of disease [18, 19, 24].

In some patients, the disease may present in adult life [25, 33]. These and other patients may have bone pains, and occasional fractures remain a part of life. Painful feet may indicate recurrent and poorly healing metatarsal stress fractures. Recurrent fractures may be the only manifestation of disease. There may be radiolucencies in the bones and pseudofractures. Pain in thighs or hips may represent femoral pseudofractures. Some patients have presented in adulthood, but give a history of rickets in childhood [6]. Recurrent arthritis and widespread calcification of articular cartilages was described in a 51-year-old woman with hypophosphatasia [18, 34]. Patients may have pseudogout resulting from periarticular deposition of calcium-pyrophosphatedihydrate (CPPD) [35]. They may have pyrophosphate (PPi) arthropathy. Early loss or extraction of teeth is common [36]. There may also be calciumphosphate deposition in ligaments [37, 38].

The term pseudohypophosphatasia [18, 26] refers to patients with otherwise typical hypophosphatasia in whom

the level of alkaline phosphatase in the plasma is normal. This may be seen in infantile, childhood, or adult forms of the disease; so it is independent of clinical severity. The fact that patients with pseudohypophosphatasia and with hypophosphatasia may be found in the same kindred indicates that this represents the vagaries of enzyme level in the serum rather than a distinct disease entity [28–32].

The major criterion for diagnosis of hypophosphatasia is an absent or extremely low activity of alkaline phosphatase in serum [39]. Alkaline phosphatase activity is also low in tissues, including bone [1]. Alkaline phosphatase activity is normal in intestine and placenta in these patients [40], indicating that the isozyme in these tissues is genetically different from that of plasma, bone, and other tissues. The defect can be demonstrated in leukocytes [41] and cultured fibroblasts [40]. There is no observable relationship between the degree of alkaline phosphatase abnormality and the clinical expression of the disease. Alkaline phosphatase is thought to be involved in the deposition of mineral crystals by the hydrolysis of pyrophosphate and other phosphate esters. Slices of rachitic cartilage from vitamin D deficient rats or patients calcify readily in normal serum or serum from patients with hypophosphatasia, but slices of cartilage from patients with hypophosphatasia do not calcify in normal serum, indicating that the defect is a local one at the site of mineralization [42].

Phosphoethanolamine (H2NCH2CH2OPO3H2) excretion in the urine [2, 43] is regularly increased in hypophosphatasia. Plasma levels are also increased. This amino acid is a useful marker for genetic studies and the detection of heterozygosity. However, its presence in the urine in other metabolic diseases of bone [44] makes it less useful in diagnosis. Normal levels decrease with age. Ranges in μmol/g creatinine are: 83–222 in those less than 15 years of age, 42–146 in 16 to 30 years; 38–155 in 31 to 41 years and 48–93 in older individuals [44]. Hydroxyproline excretion is low in hypophosphatasia [45] in contrast to vitamin D resistant rickets or hyperphosphatasia in which it is high. The concentrations of inorganic pyrophosphate in plasma and urine have been reported [46] to be increased. In the newborn phenotype the serum concentration of calcium may be elevated. Vomiting observed in some patients has been attributed to hypercalcemia. Proteinuria, casts, and impaired renal function are common in the very young.

The microscopic appearance of the bones [1, 3] resembles that of rickets. There are wide, irregular zones of proliferative cartilage and a lack of calcification of the osteoid.

GENETICS AND PATHOGENESIS

Hypophosphatasia is a rare autosomal recessive disease. Its incidence has been estimated at one in 100,000 live births. The heterozygous state can usually be demonstrated by measuring the serum activity of alkaline phosphatase [3]. However, it often is impossible to distinguish carriers from patients on the basis of activity of alkaline phosphatase. Phosphoethanolamine excretion is increased in the urine of carriers. Variability and errors in the assay of alkaline phosphatase are so common that excretion of phosphoethanolamine is probably a more reliable method for the study of families. However, it is also true that all heterozygotes studied have not had increased phosphoethanolamine excretion, even in families in which some do, therefore, it is probably well to employ both assays in order to detect all of the heterozygotes in a family [2, 24].

Prenatal diagnosis has been accomplished by the ultrasonographic appearance of the fetal head at 16 weeks [47]. The activity of alkaline phosphatase in the amniotic fluid is unreliable. In one affected fetus, it was confirmatory; in another it was normal. Prenatal diagnosis can also be accomplished by the measurement of alkaline phosphatase activity in cultured amniocytes [48].

The clinical picture of hypophosphatasia is characterized by heterogeneity. The uniformity of the age at onset and course in affected members of a single family suggests that we are dealing with a number of different genetic diseases due either to variation in alleles at a single locus or involvement of a number loci [4]. Family studies indicate clearly that the different clinical phenotypes represent different disease entities. Certainly, the severe neonatal disease and the mild juvenile disease never occur together in a kindred [27]. A form of hypophosphatasia has been reported [49] in which transmission is autosomal dominant. Clinical features were premature loss of teeth, bowed legs, and a beaten silver appearance to the roentgenogram of the skull.

Discovery of the gene (*ALPL*) that is defective in hypophosphatasia has clarified the genetics of this condition. It is clear that the classic mild hypophosphatasia, including asymptomatic people who have phosphoethanolaminuria and some with odontohypophosphatasia have dominant expression of mutation on a single allele [7, 50–52].

The more severe forms of disease are autosomal recessive. The perinatal lethal disease is common in Manitoban Mennonites in whom there is homoallelic p.G317>D [53].

The gene has been mapped to chromosome 1p36.1-34 [9] and contains 12 exons over 50Kb [54]. More than 65 mutations have been reported [10–13, 55], most of them missense. The p.E174K mutation is relatively common in Caucasians, occurring in 31 percent of people with mild hypophosphatasia [56].

TREATMENT

A number of treatments have been tried without convincing benefit. Phosphate supplementation has been employed [57]. It is usually necessary to correct craniosynostosis surgically. The usual measures are employed for the management of fractures and skeletal deformities. Dental interventions are often necessary. Nonsteroidal anti-inflammatory agents may be useful for bone pain and stress fractures.

Some patients have pyridoxine dependent seizures and respond to treatment with B_6.

Bone marrow transplantation has been without effect in severe perinatal hypophosphatasia, but transplantation of allogeneic mesenchymal stem cells cultured under osteogenic conditions and osteogenic constructs made by growing cells on porous hydroxyapatite ceramic yielded evidence of improved mineral density of bone and de novo bone formation around the constructs [58]. The treatment did not prevent the development of craniosynostosis.

Enzyme replacement therapy has been under clinical investigation in infants and children. Phase 2 clinical trials have been underway. The human gene was bioengineered by extending the C terminus and addition of a deca-aspartate sequence to target mineralizing tissue. Excellent results were obtained with subcutaneous injections in null mice [59]. Clinical trials reported to date have shown improvement in roentgenographic appearance, motor function, strength, and agility [60].

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NBAS/RALF deficiency

MAJOR PHENOTYPIC EXPRESSION

Recurrent acute liver failure (RALF) ameliorated by early antipyretic therapy, glucose infusion, and parenteral lipids; short stature; skeletal dysplasia; fractures; reduced subcutaneous fat and wrinkled skin; facial dysmorphism; optic atrophy with loss of visual acuity and colour blindness; Pelger-Huët anomaly; hypogammaglobulinemia and NK cell depletion; muscular hypotonia; no biochemical phenotype; biallelic mutations in *NBAS*.

INTRODUCTION

In Yakuts, an isolated population living in the far east of the Russian Federation, a homozygous missense mutation in *NBAS* (c.5741G>A; resulting in p.Arg1914His) was reported as the cause of a short stature syndrome in 33 affected individuals that is associated with facial dysmorphism, normal intelligence, Pelger-Huët anomaly, and optic atrophy with loss of visual acuity and colour vision, called short stature with optic atrophy and Pelger-Huët anomaly (SOPH) syndrome (MIM 614800) [1].

The discovery of patients with recurrent acute liver failure (RALF) and biallelic mutations in *NBAS* linked *NBAS* to hepatic disease [2], and the corresponding phenotype was named "Infantile Liver Failure Syndrome 2". Further patients confirmed that mutations in *NBAS* cause a wide spectrum of symptoms within a disease spectrum between the phenotypic features of SOPH syndrome and isolated RALF [3]. Retrospectively, the first patients with a multisystemic presentation due to mutations in *NBAS* have been reported in 2008 as "Developmental delay, dysmorphic features, neonatal spontaneous fractures, wrinkled skin, and hepatic failure: A new metabolic syndrome?" [4, 5].

All examined patients had reduced protein levels of NBAS in protein extracts from fibroblasts, justifying the term NBAS deficiency. The NBAS protein is functioning as a component of an endoplasmic reticulum (ER) tethering complex involved in the retrograde Golgi-to-ER transport (Figure 106.1) [2, 3, 6], and NBAS deficiency is the first disease related to a primary defect of retrograde transport. Additionally, NBAS plays a role as a mediator of the nonsense-mediated mRNA decay (NMD) pathway,

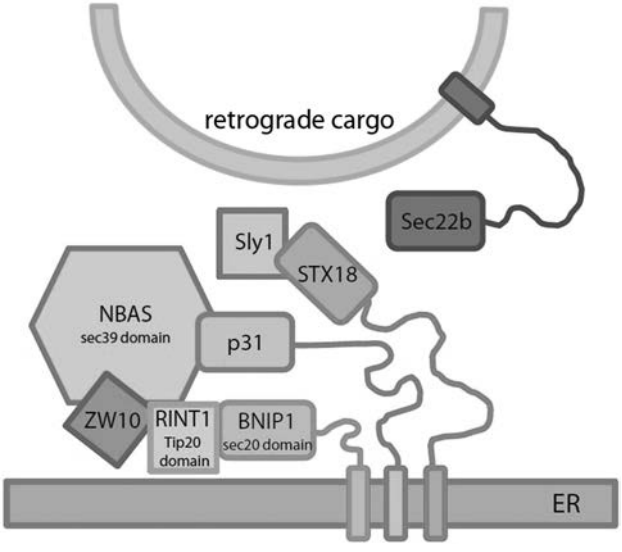

Figure 106.1 Schematic representation of the Syntaxin 18 complex. NBAS is supposed to function as a component of an ER tethering complex that interacts with the t-SNAREs p31, BNIP1, and STX18 at the ER and the v-SNARE Sec22b. This tethering complex also includes ZW10, RINT1, and Sly1. None of the components are as yet associated with a known human disorder. Mouse models of deleted p31 and RINT1 were shown to be embryonic lethal.

especially modulating genes associated with protein trafficking and ER-coupled protein modifications [7].

CLINICAL ABNORMALITIES

Mutations in *NBAS* cause a phenotypic disease spectrum with wide clinical variability, from isolated RALF to a multisystemic disease affecting liver, growth, bones, connective tissue, immune system, eye, and possibly brain.

Apart from the 33 genetically homogenous Yakuts with SOPH syndrome who were not reported to have a hepatic phenotype, 19 individuals with differing biallelic mutations in *NBAS* have been published by June 2016, and all had recurrent bouts of liver crises, which are triggered by febrile infections. Patients mostly presented with recurrent vomiting and increasing lethargy one or two days after the onset of fever, then ALAT and ASAT become massively elevated, succeeded by severe coagulopathy and mild to moderate jaundice, fulfilling the diagnostic criteria of acute liver failure (ALF). Alkaline phosphatase and gamma-GT are normal or only slightly elevated even in severe crises. Some patients had transient hepatomegaly during crises. Mild hyperammonemia and hepatic encephalopathy were also transiently observed in some individuals. Metabolic investigations did not show clear diagnostic abnormalities even during crises. Hypoketotic hypoglycemia may be present in conjunction with dicarboxylic aciduria, the latter without a disease locus specific pattern ([8] and personal observation G.F. Hoffmann). Detailed investigations of fatty acid oxidation *in vivo* were always normal [2, 3, 6]. The mean age at onset (first ALF) is 13 months, with a range from four to 24 months in most patients. In the first years of life, children are very prone to infections and crises can occur with every illness and even vaccinations. Some parents, therefore, kept their children from going to kindergarten school in the first years. Crises become less frequent and less severe with age but are not restricted to childhood. Severity of the hepatic phenotype in NBAS deficiency ranges from death in acute liver failure, RALF with up to ten episodes during childhood, to intermittently elevated activities of ALAT and ASAT without coagulopathy. Liver biopsies typically show microvesicular steatosis but no fibrosis. Immunohistochemical analysis of NBAS was much less intense or absent in comparison to controls [3]. Complete recovery is typical, but ALF crises can be fatal [3, 5]. Prompt intervention by early antipyretic therapy, glucose infusion, and parenteral lipid can ameliorate and shorten the crises. In the interval, liver function and transaminases are normal, but one patient is known to the authors that had consistently elevated ALAT and ASAT.

Some individuals had episodes of hypoglycemia, mostly associated with liver crises but also in a period following an acute liver crisis. It has remained unresolved, whether this can be explained by unspecifically hindered liver function or whether other mechanisms are responsible.

Short stature is one of the most frequent findings in NBAS deficiency (Figure 106.2). The affected Yakuts had a mean SDS of height of −4.44 in females and −3.16 in males [1], in our study population, mean height was −2.64 SDS [3], the most severe case known to us however had a severe growth retardation with −6.10 SDS (despite a genetically determined body height of 0.89 SDS) (Figure 106.3). The severity of growth retardation seems to be associated with further skeletal features, such as thin bones and epiphyseal dysplasia with multiple phalangeal pseudo-epiphyses, reminiscent of a

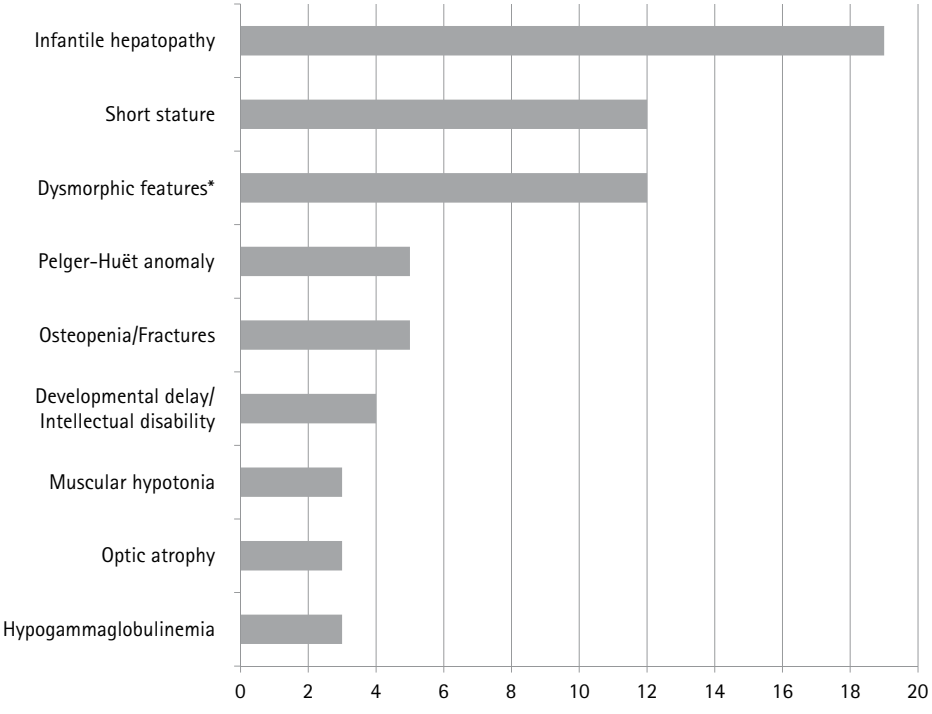

Figure 106.2 Clinical signs and symptoms of NBAS deficiency. The symptoms are listed according to the frequency of their report in published patients, taking together 19 patients from 16 families [3, 7, 8]. The Yakut individuals with SOPH syndrome are not included. All these 19 patients have infantile hepatopathy, and in all but one, onset was before two years of age. Short stature and dysmorphic features are the most frequent extrahepatic findings. *"Dysmorphic features" also includes minor signs, such as hypotelorism (see Figure 106.4).

Figure 106.3 Next to infantile hepatopathy, short stature is the most frequent finding in NBAS deficiency. At the age of 4 9/12 years, this girl had a body height of 83.5cm (-6.10 SDS). Apart from severe growth retardation, she has bilateral optic atrophy with a visual acuity of 0.1 and convergent strabismus.

disturbance in bone mineralization. Two patients were found to have small cervical vertebrae (C1, C2) causing cervical instability [8]. Large fontanels with delayed closure, short neck, and abnormal thoracic configuration have also been described. There may be frequent or spontaneous fractures, even from the neonatal age on [5].

Facial characteristics have been described in most individuals, including long facies, flat cheekbones, hypotelorism, prominent eye brows, pointed chin, and thin upper lip (Figure 106.4). Reduced periorbital fat leads to the aspect of proptosis in some individuals and some have a prominent forehead. A progeroid appearance may be present due to loose, wrinkled skin and reduced subcutaneous fat [5, 8] (Figure 106.5). However, in many patients, the physical appearance is unsuspicious and even completely normal [3].

Most patients reported have normal intelligence [1, 3, 8], however, some individuals had developmental delay and muscular hypotonia [5], and two subjects had epilepsy [3]. Brain magnetic resonance imaging (MRI) revealed nonspecific patterns of mild brain atrophy with mild supratentorial white matter deficit and/or mild atrophy of

Figure 106.4 Facial characteristics of five individuals with NBAS deficiency: long facies, flat cheekbones, prominent eye brows, hypotelorism, narrow nasal bridge, pointed chin, and thin upper lip.

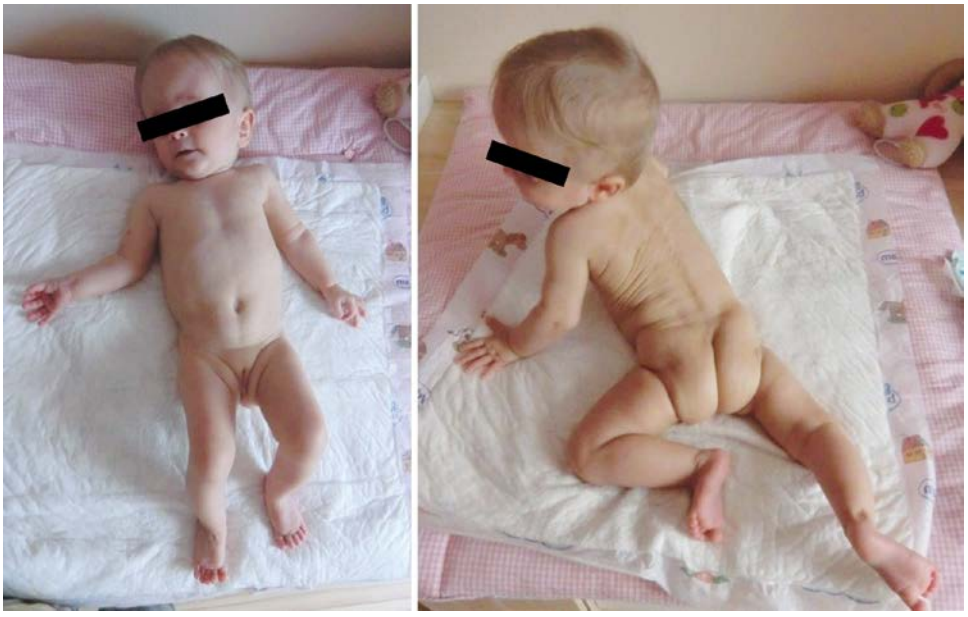

Figure 106.5 A girl with NBAS deficiency at the age of 1 year (same patient as shown in Figure 106.3). She has a prominent forehead and reduced periorbital fat gives the impression of proptosis. Subcutaneous fat is also reduced and skin is loose and wrinkled.

the superior vermis in six of eight patients [3], whereas within the Yakut cohort it was normal in 18/24 individuals, three had slight cerebellar atrophy and one had each Dandy-Walker malformation, cyst at internal capsule, and empty sellae [1].

Pelger-Huët anomaly, which is characterized by a hypolobulation of granulocytes, was present in all affected Yakut individuals, but only in some of the other patients [1, 3, 8]. There may be hypogammaglobulinemia and reduced natural killer cells, which can be associated with frequent infections [3, 8]. Possibly, there is an association with autoimmune diseases, as out of 14 patients one had celiac disease and one inflammatory bowel disease (Crohn's disease) [3].

All of the affected Yakuts had optic nerve atrophy with a mean visual acuity of 0.23 ± 0.21 and all but one had complete achromatopsia, caused by nonprogressive cone dysfunction. Mean onset of visual loss was 4.3 years of age [1]. Most other *NBAS*-deficient individuals had normal visual function and no optic nerve atrophy or a very mild ophthalmologic phenotype [3], but few are severely affected resembling the Yakuts' phenotype. Myopia, strabismus, and hypermetropia have also been observed.

GENETICS AND PATHOGENESIS

Transmission of the disease is autosomal-recessive. The prevalence is unknown. Within a group of 15 unrelated individuals with RALF, six individuals from five families have been identified with biallelic mutations in *NBAS* [2]; this raises the possibility that NBAS deficiency is a relatively frequent cause of RALF and possibly also single ALF.

NBAS (neuroblastoma amplified sequence, GenBank: NM_015909.3, MIM: 608025), located on the short arm of chromosome 2 (2p24.3), was initially described as a gene coamplified with N-Myc in neuroblastoma tumor cells but its role in neuroblastoma has remained unclear [9]. Apart from the homozygous c.5741G>A mutation found in the Yakut population with SOPH syndrome, 25 different mutations in *NBAS* have now been identified in 19 patients from 16 families, including missense mutations, in-frame deletions of one amino acid, stop mutations, frameshift mutations, and splice site mutations. All individuals carry at least one missense mutation or a deletion of a single amino acid on one allele. In two families, patients had homozygous mutations, c.409C>T (p.Arg137Trp) respectively c.2708T>G (p.Leu903Arg) [3, 5]. All other mutations were compound-heterozygous. Most mutations were private. Two mutations, c.2827G>T (p.Glu943*) and c.3164T>C (p.Leu1055Pro), have each been found in three unrelated individuals, while three mutations have each been described in two unrelated patients: c.409C>T (p.Arg137Trp), c.686dup (p.Ser230Glnfs*4), and c.2708T>G (p.Leu903Arg) [2, 3, 5, 8].

In patient fibroblasts, there was a reduction of NBAS levels to 18 percent to 36 percent in comparison to controls [2]. Also protein levels of p31, an interaction partner of NBAS, are decreased in patients' fibroblasts. When fibroblasts are challenged by a temperature shift from 37 to 40°C, NBAS and p31 levels further decrease, and cells showed a reduced growth rate compared to controls and it was concluded that raised temperature itself may be the starting point of a derailment leading to ALF [3].

The protein NBAS is part of a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, the syntaxin 18 complex [6]. SNAREs mediate the docking and fusion of transport vesicles with target membrane and can be bound to transport vesicles (v-SNARE) or target membranes (t-SNARE). Within the syntaxin 18 complex, NBAS interacts with several partners, including the t-SNARE p31 (see Figure 106.1)

[6]. The concomitant reduction of p31 emphasizes a role of NBAS within the syntaxin 18 complex. There is evidence that NBAS as part of the syntaxin 18 complex is involved in the retrograde Golgi-to-ER transport [3, 6]. ER morphology in hepatocytes from patients' liver biopsy samples as determined in transmission electron microscopy was found to be abnormal with increased and enlarged ER [3]. Altered ER induces ER stress and apoptosis in p31-depleted cells [10], and in NBAS-mutant fibroblasts expression of genes involved in ER stress response is significantly increased [2]. Silencing of the NBAS homolog in the plant model (Nicotiana benthamiana) causes ER stress, disruption of the ER network, and apoptosis [11]. It was therefore hypothesized that in NBAS-mutant patients fever-dependent ALF may be the common final path of ER stress-induced liver cell apoptosis, set off by temperature-dependent NBAS and p31 depletion [3].

Apart from its role as a SNARE component, the system has been shown to act as an important mediator of the NMD pathway in human cells, zebrafish embryos and C. elegans [7]. The NMD pathway is responsible for selectively degrading mRNAs harboring premature termination codons but also regulating the abundance of cellular RNAs. The majority of genes regulated by NBAS are associated with protein trafficking and ER-coupled protein modifications [7], linking a role of NBAS in the NMD pathway to intracellular trafficking. Furthermore, genes associated with bone mineralization and development have been shown to be regulated via NBAS, and it is tempting to discuss that this may contribute to the skeletal phenotype of affected patients [7].

As there is no specific diagnostic laboratory marker, diagnosis of NBAS deficiency relies on genetic testing. The analysis of NBAS protein levels via immunohistochemistry of liver biopsy or Western blot analysis of fibroblasts may help to validate genetic findings of unknown significance.

TREATMENT

There is no established treatment for NBAS deficiency. However, it has been observed in six patients that liver crises can be ameliorated and shortened through early and consequent antipyretic therapy and induction of anabolism through IV glucose and parenteral lipids, especially when begun early in the course of ALF. Early and effective antipyretic therapy may even prevent liver crises. Patients should be supplied with an emergency card, letter, or bracelet containing instructions for emergency measures and phone numbers. One patient was liver transplanted at the age of three years. She has not suffered any further crises since then [3]. Fixation surgery may become necessary in case of cervical instability due to hypoplastic cervical vertebrae [8]. In patients with hypogammaglobulinemia, immunoglobulin replacement should be considered. This can reduce frequency of infections, hereby avoiding triggers of liver crises [8].

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α_1-Antitrypsin deficiency

MAJOR PHENOTYPIC EXPRESSION

In infancy and childhood: hepatic disease with conjugated hyperbilirubinemia, hepatocellular damage presenting in a neonatal hepatitis pattern or isolated hepatomegaly. In adulthood: emphysema of early onset. Each has deficiency of α_1-antitrypsin.

INTRODUCTION

α_1-Antitrypsin (AT) deficiency was discovered by Laurell and Eriksson [1] in 1963, when they reported five patients in whom the α_1-globulin band was missing on agarose gel protein electrophoresis. Three had emphysema, and so did nine of 14 other patients with α_1-AT deficiency [2]. The protein itself had been isolated in 1955 by Shultze and colleagues [3], who recognized its function as the major trypsin inhibitor of serum and the major component (90 percent) of the α_1-globulin fraction [4]. It was evident early that partial deficiency was transmitted in an autosomal dominant manner, and that those with severe deficiency were homozygotes [5]. Extensive polymorphism of α_1-AT was recognized first by Fagerhol [6] on electrophoresis on starch gels. The current method of choice is isoelectric focusing on polyacrylamide gel [7], and 75 variants have been recognized. Examination of the DNA for restriction fragment length polymorphism (RFLP) indicated even greater variation [8, 9].

A classification system has been developed [8] in which the variant proteins are designated as protease inhibitor (PI) types. More than 70 PI types are known, most of them with normal α_1AT. The normal variant was designated PIMM. The classic deficiency phenotype in which serum AT activity is about 15 percent of normal is PIZZ. PIMZ individuals are heterozygotes. PISS is the second most common form of deficiency.

The relationship of α_1-AT deficiency to hepatic disease in infancy and childhood was discovered by Sharp and colleagues in 1969 [10].

The disease is a model in which an abnormal glycoprotein synthesized in the liver is not released from the hepatocyte into the circulation. The disease provides a model for the understanding of the processing of correctly and incorrectly folded glycoproteins in the endoplasmic reticulum [11]. The (SERPINA1) gene has been cloned and localized to chromosome 14q32.1 [12]. A number of deficiency mutations has been defined. The Z allele contains a G to A change in exon 5 that changes glutamic acid 342 to lysine (E342 K) [13, 14].

The relationship of protease and elastin has led to understanding of the pathogenesis of emphysema not only in this common disease, but in other nongenetic forms of emphysema. The gene has been isolated. Recombinant techniques have made abundant supplies of α_1-AT for protein replacement therapy [15, 16], and gene replacement has been done in transgenic animals [17, 18]. In addition, the relationship between cigarette smoking and the occurrence of emphysema in individuals that have inherited this susceptibility [19] provides an interesting example of the interaction of genetics and environment, and the development of emphysema in non-α_1-AT deficient smokers has provided a strong scientific argument against smoking [20].

CLINICAL ABNORMALITIES

Hepatic manifestations of α_1-AT deficiency were first recognized by the detection of the deficiency in 14 patients with liver disease [10]. All were PIZZ homozygotes. These

patients developed specific manifestations of liver disease in the first year of life with early cholestasis that resolved by 6 months of life, but elevated serum levels of hepatocellular enzymes and hepatomegaly persisted. All but one developed cirrhosis. It has since become evident that most PIZZ individuals do not have severe liver disease. Some 10 percent of PIZZ infants have neonatal cholestasis [21], and about half of PIZZ infants who appear normal have abnormal serum levels of aminotransferases. The most frequent hepatic presentation is with a neonatal hepatitis syndrome [22]. These infants have conjugated hyperbilirubinemia and hepatomegaly. Vomiting may be projectile. Failure to thrive is common; some have low birth weights. Some have splenomegaly. There may be dark urine, indicating the presence of bilirubin. Bilirubinemia is a uniform indicator of cholestatic disease because indirect bilirubin is bound to albumin and not present in urine. Urinary bile acid concentration may be elevated [23].

This disorder is a major cause of the neonatal hepatitis syndrome. It has been found in 14–29 percent of such infants [24]. Bleeding may occur as a result of vitamine K deficiency. Occasionally in such an infant, there are acholic stools and the picture may simulate extrahepatic biliary atresia [25]. The diagnosis of α_1-AT deficiency should obviate the usually demanding work-up for this disorder. In α_1-AT deficiency, the jaundice usually clears spontaneously by 7 months-of-age [26].

Another presentation is with transient symptoms of liver disease occurring with intermittent infection or appendicitis at 2 years or later in a previously asymptomatic child [26]. In another group of patients, hepatomegaly has been found in childhood without a history of neonatal jaundice. Others may present first in childhood with what appears to be acute hepatitis.

In many patients, once the jaundice has subsided, clinical manifestations of liver disease do not recur, and ultimately serum levels of aminotransferases become normal [22]. A small number go on to develop chronic cirrhosis (Figure 107.1). Cirrhosis and early death have been reported in two percent of children with PIZZ and 14 percent of those with hepatic manifestations in infancy [27]. These patients may have spider telangiectases as early as 2 years-of-age. They may develop portal hypertension and esophageal varices. A number have died of this or other complications of cirrhosis during childhood or adolescence, even in infancy, and certainly after the development of cirrhosis. Ascites may be present with or without hypoalbuminemia and edema elsewhere.

We have observed a 10-month-old patient with α_1-AT deficiency who presented with ascites that appeared on paracentesis to be chylous. Its protein content was 4.1 g/dL, and the serum albumin concentration was 3.5 g/dL. This is the so-called pseudochylous ascites in which some patients with chronic liver disease have lactescent fluid. Triglyceride content is not high, and this fluid can be distinguished by adding petroleum ether and shaking well, under which circumstances the triglyceride in true chylous fluid dissolves and the fluid becomes clear. Protein content has been used to distinguish exudative from transudative processes, but the range of protein content in liver disease is too great to permit this distinction. Coagulation factors may be reduced, and there may be clinical bleeding, especially gastrointestinal. Pruritis may be present in infancy, or may develop later. We have observed hyperammonemia and hepatic encephalopathy. Cirrhosis has also been observed in a number of adults who had a history of neonatal hepatitis or liver disease in childhood [28]. A few of these patients were found to have hepatomas on biopsy. Levels of α-fetoprotein are not usually elevated in these patients with hepatomas. Hepatocellular carcinoma has been observed.

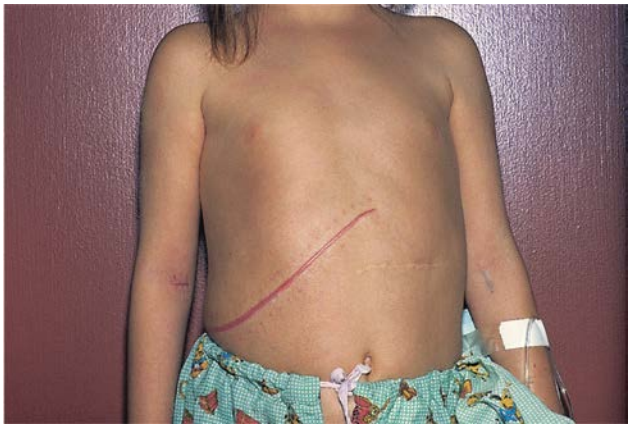

Figure 107.1 J.P.: A 7-year-old girl with α_1-antitrypsin deficiency who presented with hematemesis. She had been found to have hepatomegaly at 2 years-of-age and biopsy revealed cirrhosis. She had two spider telangiectases on the arms and a large healed incision over the liver, which was palpable 3 cm below the costal margin. The spleen was palpated at 8 cm. Endoscopy revealed esophageal varices.

Pulmonary disease is the most common expression of the PIZZ phenotype [29] (Figure 107.2). As many as 90 percent develop emphysema. It is classically early in onset, occurring at 20–40 years of age in smokers and 55 in nonsmokers [5, 30, 31]. It is referred to as chronic obstructive pulmonary disease or COPD. The earliest symptom is dyspnea on exertion. Cough develops in about half of the patients, and recurrent pulmonary infections are common. On examination, the patient may be thin, but the diameter of the chest is increased. Breath sounds are diminished, and the chest film reveals hyperinflation, especially in the bases. The diaphragms may be flattened. Pulmonary function tests are typical of severe emphysema consistent with a loss of pulmonary elastic recoil. Total lung capacity is impaired, as is residual volume. Air flow is limited, and diffusion capacity and maximum transpulmonary pressure are reduced. Mild hypoxemia at rest may increase with exercise. Hypocarbia and respiratory alkalosis may be associated with mild pulmonary hypertension. Electrocardiograms may show chronic strain on the right heart with right axis deviation and right atrial hypertrophy. There may be a right bundle branch block.

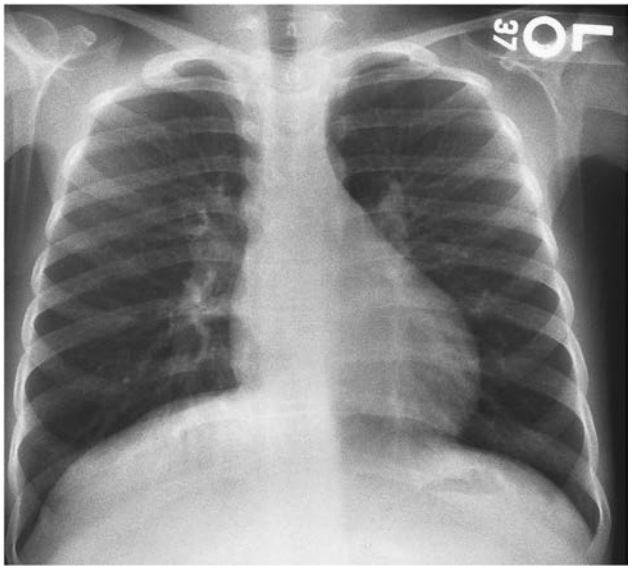

Figure 107.2 Roentgenogram of the chest of C.T., a 10-year-old girl with α_1-AT deficiency. There were no pulmonary symptoms, but bronchial markings were increased as linear densities, there was hilar prominence, and there was evidence of hyperinflation.

The early and more prominent involvement of the lower lobes [31] is in contrast to the preferential involvement of the upper lobes in acquired emphysema. Angiography reveals decreased arborization in the lower lobes in α_1-AT deficiency [32] and radionuclide scanning shows diminished perfusion in these areas [33].

Chronic pulmonary disease in PIZZ children is not common, but some do have clinical or roentgenographic evidence of abnormality (Figure 107.2) [21, 22, 34], and more have abnormalities detectable by pulmonary function tests. Children with PI null variants may have severe emphysema early in life.

The relationship between smoking and the development of emphysema in this disease has led to the concept of genetic predisposition, in which the defective gene alone is not sufficient to produce the disease, at least by a certain age; noxious elements in the environment combine with the predisposition to yield illness. Smoking influences not only the age of onset of emphysema but the rate of its progression [35–38]. The emphysema is always associated with progressive decrease in lung function. In terms of survival, only 18 percent of PIZZ males who smoke are alive at 25 years-of-age, while the figure is 65 percent for nonsmokers. The comparable figures for females are 30 percent and 98 percent [35]. Smoking in adolescence is particularly effective, because maximal pulmonary function is not achieved [39].

The pathology of the lung has indicated that emphysema results from a destructive process involving the alveoli. Electron microscopy reveals extensive destruction of alveolar septal walls with loss of alveolar structure and large air-filled spaces.

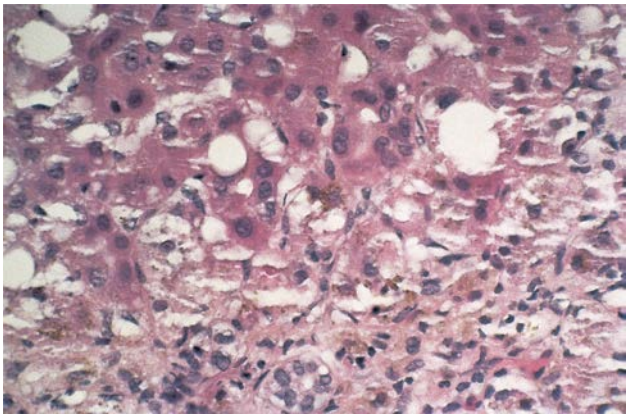

Figure 107.3 H- and E-stained biopsied liver of a patient with α_1-AT deficiency. Hepatocytes contain eosinophilic globular inclusions. These are especially prominent in periportal areas. In addition, there is fibrosis. (Illustrations in Figures 107.3–107.7 kindly provided by Dr. Henry Krous of the Children's Hospital and Health Center, San Diego, CA.)

The pathology of the liver provided early insights into the molecular pathogenesis of the disease. The distinctive feature is the presence of globules of α_1-AT in the cytoplasm of the hepatocytes [22, 40] (Figures 107.3 and 107.4). They are present at birth and enlarge with age. They are most prominent in the periportal regions. They stain positively with PAS stain after treatment with diastase (Figure 107.4), and positively with Oil Red 0 (Figure 107.5). In addition, the changes of chronic hepatic disease are nonspecific, but progressive. During the infantile cholestatic stage, there is proliferation of bile ducts, fibrosis, some accumulation of fat and occasional giant cells. Later, there is typical cirrhosis. The material has been documented to be α_1-AT by immunofluorescence studies with antibody against α_1-AT (Figure 107.6). In the electron microscopic picture, the accumulated amorphous-appearing material is localized to the lumen of the endoplasmic reticulum

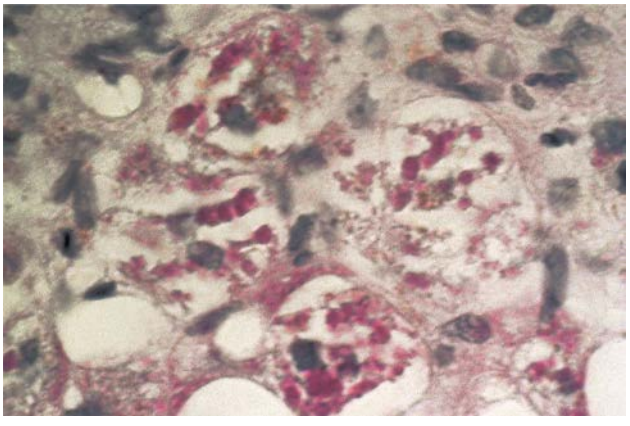

Figure 107.4 PAS stain after treatment with diastase reveals the bright pink PAS-positive inclusions.

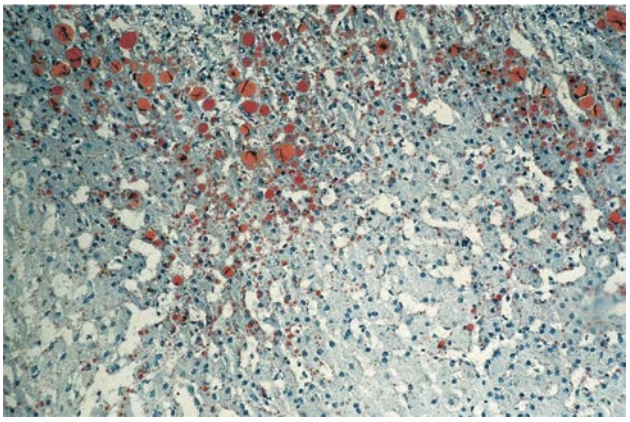

Figure 107.5 Oil Red O stain indicates some increase in fat.

(Figure 107.7) [22, 40]. In patients with cystic fibrosis, the Z phenotype is a risk factor for the development of severe liver disease with cirrhosis and portal hypertension [41].

In addition to disease of the lungs, membranous glomerulonephritis has been observed histopathologically, and some of these patients have had signs of renal dysfunction [42]. Some evidence of glomerular disease is common at post-mortem examination in patients dying of liver disease. Immune complex disease is suggested by immunofluorescent evidence of α_1-AT along with immunoglobulins and C3 on the glomerular basement membrane. A variety of inflammatory disorders have been associated with heterozygosity for the Z allele, including rheumatoid arthritis [43] and uveitis [44]. Severe panniculitis has been reported in 22 homozygotes [45, 46].

The diagnosis of α_1-AT deficiency is by quantitative analysis of the content of α_1-AT in serum. Immunologic techniques are the best. The normal range is 20–50 μmol/L, and in the Z variant, it is 3–6 μmol/L. Patients with concentrations under 40 percent of normal should be PI typed.

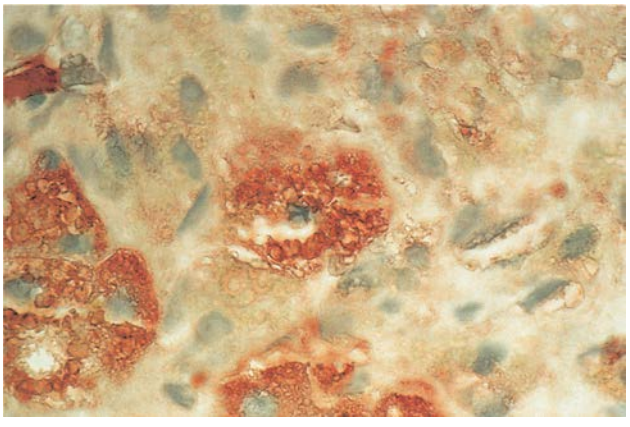

Figure 107.6 Immunoperoxidase-labeled anti-α_1-AT antibody identifies the stored material as α_1-AT.

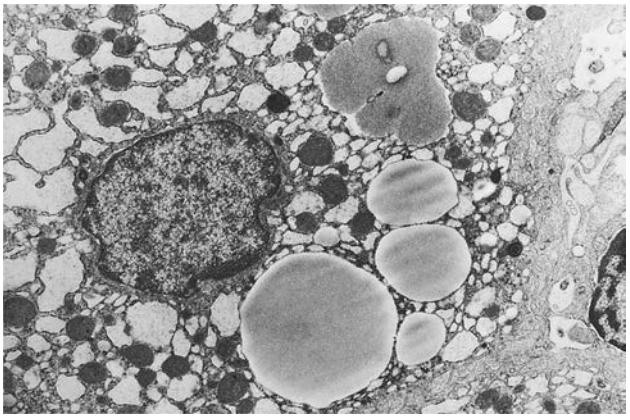

Figure 107.7 Electron microscopy shows the hepatic inclusions to be membrane-bound.

GENETICS AND PATHOGENESIS

The normal α_1-AT phenotype is PIMM, and the classic deficient phenotype is PIZZ [9]. M and Z are codominant autosomal alleles. The heterozygotes are PIMZ. In the PIZZ individual, serum α_1-AT activity is 15 percent of normal. The frequency of the PIZZ phenotype in Sweden, where the disease was discovered, is approximately one in 1500. In the United States, it is one in 6000, and it is more common in those from Europe than from Africa or Asia [20]. The gene frequency for PIZZ in Sweden was reported to be 0.026 [47]. In the United States, it is 0.01, while the normal M allele approximates 0.95 [25] and the S allele is 0.03. The PISS homozygote has about 60 percent of normal α_1-AT activity. Among the many other variants [8], most have normal activity. Exceptions are PIII at 68 percent and PIPP at 30 percent of normal [48, 49] and the null variants (PI null) in which there is no detectable α_1-AT in serum [8]. Hepatic disease of prenatal origin has been reported in a PIZ null heterozygote [50].

The α_1-AT protein is a glycoprotein with a single polypeptide chain. Its molecular weight is 52 kDa, and its carbohydrate content of 12 percent contains a number of sialic acid residues. The protein synthesized in the liver is longer, containing a signal peptide and an N-terminal methionine [51].

The gene for α_1-AT is located on chromosome 14 at position q32.1 [12, 52]. It is 12.2 kb in length and contains 4 exons in a 1434 bp coding region [51]. In classic PIZZ α_1-AT deficiency, a single nucleotide substitution of adenine for guanine codes for a lysine instead of glutamic acid in the M protein (Table 107.1) [13, 53]. Oligonucleotide probes have been made which recognize the Z and M

Table 107.1 Site of the defect in classic α_1-AT deficiency

PI phenotype	Gene	Protein	Amino acid position
M	GAG	Glutamic acid	342
Z	AAG	Lysine	342

sequences and can be used for diagnosis. This is particularly important for prenatal diagnosis, because prior to their development prenatal diagnosis was available only through fetal blood sampling. In the S variant, a glutamic acid at position 264 is changed to valine. Null alleles represent a heterogeneous group of mutations in which a variety of different mechanisms lead to an identical phenotype [54]. Prenatal diagnosis has been carried out in two pregnancies at risk for the ZZ disease by oligonucleotide hybridization to DNA of cultured amniocytes [55]. Both fetuses were found to be MZ heterozygotes. Parental PI typing is essential for prenatal diagnosis to be sure both parents have the Z allele, and that there is no null or rare deficiency allele. Currently, the method of choice is to employ PCR amplification of exon 5 and detection of the Z mutation by oligonucleotide probes labeled with 32P [56] or with biotin [57].

α_1-AT is normally synthesized in the rough endoplasmic reticulum of hepatocytes [8]. Cultured hepatocytes secrete α_1-AT; and *in vivo* α_1-AT is secreted from the liver into the blood. In the Z variant, and other variants in which there is deficiency of α_1-AT, the α_1-AT protein is synthesized normally and levels of mRNA are normal, but the nature of the variant protein is such that it cannot be transported out of the endoplasmic reticulum. The Z variant protein isolated from the liver functions normally [58]. The failure of transport is thought to result from changes in the three-dimensional structures which interfere with normal folding and lead to local aggregation. In the normal protein, the glutamic acid at 342 is thought to form a salt bridge with lysine at 290. The substitution of the lysine at 342 would abolish this salt bridge. Support for this hypothesis was obtained by site-directed mutagenesis in which the lysine 290 in the Z protein was changed to glutamic acid, which would re-establish the salt bridge, and the resultant protein was secreted normally [59]. On the other hand, disruption of the salt bridge by changing the wild type lysine 290 to glutamic acid was followed by near normal secretion of the protein, suggesting that tertiary structure is more important than the salt bridge [60]. Aggregation of the Z protein with itself results in the aggregations that form the hepatic inclusions. There is a mobile reactive center loop in the Z protein, which locks into that of another molecule, causing dimerization [61]. This is temperature-sensitive; so, an increase in body temperature with fever would be expected to increase aggregation.

The processing of α_1 AT in the endoplasmic reticulum is aided by the transmembrane chaperone calnexin which is involved in the degradation of abnormally folded proteins [62]. Misfolded proteins are dislocated to the cytosol and degraded by the ubiquitin-proteasome system, known as endoplasmic reticulum-associated degradation (ERAD) [11]. A null variant of α_1-AT, Hong Kong, is a substrate for ERAD. Wild type AT was transported to the Golgi, and its carbohydrates were modified into complex glycans. In contrast, the stay in the ER of the null protein was prolonged and had protracted interaction with calnexin. Retained incompetent glycoproteins became substrates for the α-mannosidase I that tags ERAD candidates with mannose-8-glycans, which are then subject to accelerated degradation [63].

Introduction of the Z variant human gene into mice led to accumulation of the mutant human protein in mouse liver, and this was followed by hepatic necrosis and inflammation [64].

α_1AT in the circulation is protective of the lung because it is a very effective inhibitor of elastase and other proteolytic enzymes released from neutrophils and macrophages during the inflammatory process [65, 66]. The inactivation of elastase protects the elastic fibers of the lung [66, 67]. A growing body of evidence indicates that emphysema represents an imbalance between protease and antiprotease activity in the lung. Elastase itself produces emphysema in experimental animals, as it consumes pulmonary elastin. Furthermore, cigarette smoke inactivates α_1-AT, providing a mechanism for the next most frequent cause of emphysema [68].

TREATMENT

Treatment for hepatic disease is primarily supportive. This includes supplementation with vitamin K and vitamin D. Most patients do not go on to cirrhosis. In those that do, cholestyramine may be effective in the management of pruritis. Portacaval or splenorenal shunt may relieve esophageal varices [69]. Transplantation of the liver is curative for advanced hepatic disease [70]. The patient is then left with the prospect of pulmonary disease.

Replacement therapy has been undertaken with intravenous α_1-AT in pharmacological amounts, and protective levels have been obtained in lung fluid as well as serum [71, 72]. The product has been licensed in the United States as an Orphan Drug. Recombinant techniques have made available ample quantities [15, 16]. Treatment requires weekly intravenous administration, and it is expensive. Analysis of data indicated that the rate of decline of forced expiratory volume was reduced, and so was mortality [38]. Trials were begun with aerosolized α_1-AT [73], because so little intravenously administered protein reaches the lungs. Gene therapy has been accomplished in transgenic mice [17, 18].

Avoidance of smoking has been shown to make for an impressive improvement in morbidity and life expectancy [19]. This, and the frequency of the gene, led to a newborn screening program in Sweden in which 200,000 newborns were screened for α_1-AT deficiency [74, 47], but the program was stopped because of unexpected psychological effects. Parents assumed that α_1-AT deficiency posed an immediate serious threat to the health of the child [75], and these negative feelings persisted for 5–7 years [76]. The lesson is that newborn screening requires a considerable effort at public education, and this may be particularly true if the effects of the disease are long delayed. Nevertheless, screening for α_1-AT deficiency combined with a comprehensive program aimed at the avoidance of smoking could markedly decrease morbidity.

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Appendix: Differential diagnosis of clinical phenotypes

CONTENTS

ACIDOSIS, HYPERCHLOREMIC

Diarrhea
- Acrodermatitis, enteropathica
- Infectious
- Lactase deficiency
- Sucrase deficiency

Renal tubular acidosis (RTA)
- Cystinosis
- Fanconi syndrome
- Galactosemia
- Glucose, galactose malabsorption
- Hepatorenal tyrosinemia
- Mitochondrial electron transport defect
- Osteopetrosis and RTA
- Topiramate

ALOPECIA

Acrodermatitis, enteropathica
An (hypo) hidrotic ectodermal dysplasia
Biotin deficiency
Cartilage hair hypoplasia
CHILD syndrome (unilateral)
Congenital erythropoietic porphyria
Conradi-Hünermann syndrome
Methylmalonic academia
Multiple carboxylase deficiency – holocarboxylase synthetase and biotinidase deficiencies
Porphyria cutanea tarda type II
Propionic academia
Trichorrhexis nodosa-argininosuccinic aciduria
Vitamin D-dependent rickets-receptor abnormalities

ANGIOKERATOMAS

Aspartylglucosaminuria
Fabry disease
Fucosidosis
Galactosialidosis
GM_1 gangliosidosis
Mannosidosis
Sialidosis

APPARENT ACUTE ENCEPHALITIS

Alpers disease
Biotin-responsive basal ganglia disease or thiamine-responsive encephalopathy
Glutaric aciduria type I
Mitochondrial disorders (especially POLG mutations, NARP)
Propionic acidemia

ARTHRITIS

Alkaptonuria
Farber disease
Gaucher type I
Gout-HPRT deficiency; PRPP overactivity
Homocystinuria
I cell disease
Lesch-Nyhan disease
Mucolipidosis III
Mucopolysaccharidosis I; II

BLACK PIGMENT DEPOSITION

Alkaptonuria – ochronosis
Minocycline

BLEEDING TENDENCY

Abetalipoproteinemia
α1-Antitrypsin deficiency
Congenital disorders of glycosylation (CDG)
Chediak-Higashi syndrome
Fructose intolerance
Gaucher disease
Glycogenoses types I and IV
Hermansky-Pudlak syndrome
Peroxisomal disorder
Tyrosinemia type 1

CALCIFICATION OF BASAL GANGLIA

Albright syndrome
Bilateral striato-pallido-dentate calcinosis
Carbonic anhydrase II deficiency
Cockayne syndrome
Dihydrofolate reductase (DHFR) deficiency
Down syndrome
Familial progressive encephalopathy with calcification of the basal ganglia (Aicardi-Goutieres syndrome)
GM_1 gangliosidosis
Hallervorden-Spatz disease
Krabbe leukodystrophy
Lipoid proteinosis
Microcephaly and intracranial calcification
Mitochondrial cytopathies, MELAS, MERRF
Neurofibromatosis
Panthothenate kinase-associated neurodegeneration (PKAN, formally Hallervorden–Spatz disease)
Pterin defects
- Dihydropteridine reductase deficiency
- GTP cyclohydrolase I deficiency
- 6-Pyruvoyletetrahydropterin synthase deficiency
- Sepiapterin reductase deficiency

Spondyloepiphyseal dysplasia

CARDIOMYOPATHY

Congenital muscular dystrophy
Danon disease
Disorders of fatty acid oxidation
Electron transport chain abnormalities
Fabry disease
Glycogenosis type III
Hemochromatosis
D-2-Hydroxyglutaric aciduria
3-Methylglutaconic acidurias (Barth syndrome, Sengers syndrome, TMEM70 deficiency, dilated cardiomyopathy with ataxia (DCMA) with mutations in DNAJC19 gene)
Mucopolysaccharidoses
α-Mannosidosis
Pompe disease

CARNITINE ESTERS

C3 carnitine, elevated
- Propionic acidemia
- Methylmalonic acidemia

C4-carnitine, elevated
- Ethylmalonic encephalopathy (C-5 may also be increased)
- Glutamate formiminotransferase (FTCD, MIM 229100) deficiency
- IsobutyrylCoA dehydrogenase deficiency
- SCAD (short chain acylCoA dehydrogenase) deficiency (C-5 may also be increased)

C4OH, elevated
- M/SCHAD (medium/short) chain acylCoA dehydrogenase deficiency

C5-carnitine, elevated
- Isovaleric acidemia
- MethylbutyrylCoA (short branched chain) dehydrogenase deficiency

C5OH carnitine, elevated
- 3-Hydroxy-3-methylglutarylCoA lyase deficiency
- 2-Oxothiolase deficiency
- 3-MethylcrotonylCoA carboxylase deficiency (including maternal)
- 2-Methyl-3-hydroxybutyrylCoA dehydrogenase deficiency
- Mitochondrial acetoacetylCoA (3-oxo)thiolase deficiency
- Multiple carboxylase deficiency (biotinidase, holocarboxylase synthetase)
- Propionic acidemia (C3 always higher)

C8, C6, C10, elevated
- MCAD deficiency

C14:1, elevated
- VLCAD deficiency

C16OH, C18:1OH, elevated
- LCHAD deficiency

CATARACTS – LENTICULAR OPACITY

Cerebrotendinous xanthomatosis
Cholesterol biogenesis disorders
Dystroglycanopathies
Electron transport chain disorders
Fabry disease
Galactokinase deficiency
Galactosemia (classical)
Homocystinuria
Hyperferritinemia-cataract syndrome
Hyperornithinemia (ornithine aminotransferase deficiency)
Lowe syndrome
Lysinuric protein intolerance
Mannosidosis
Multiple sulfatase deficiency
Neonatal carnitine palmitoyl transferase (CPT) II deficiency
Δ^1 Pyrroline-5-carboxylate synthase deficiency
Peroxisomal disorders
Zellweger syndrome

CEREBRAL CALCIFICATION

Abnormalities of folate metabolism
Adrenoleukodystrophy
Aicardi-Goutieres syndrome
Biopterin abnormalities
Biotinidase deficiency
Carnitine palmitoyltransferase II deficiency
Dihydrofolate reductase (DHFR) deficiency
Cockayne syndrome
GM_2 gangliosidosis
Hypoparathyroidism
Kearns-Sayre syndrome
Krabbe disease
L-2-Hydroxyglutaric aciduria
Mitochondrial disorders (including MELAS)
Osteopetrosis and renal tubular acidosis (carbonic anhydrase II deficiency)

CEREBRAL VASCULAR DISEASE

Fabry disease
Familial hypocholesterolemia
Homocystinuria
Menkes disease
Methylene tetrahydrofolate reductase deficiency

CEREBROSPINAL FLUID LYMPHOCYTOSIS

Aicardi-Goutieres syndrome

CEREBROSPINAL FLUID PROTEIN ELEVATION

Congenital disorders of glycosylation CDG
L-2-Hydroxyglutaric aciduria
Kearns-Sayre
Krabbe disease
MELAS (mitochondrial encephalomyopathy, lactic acidemia, and stroke-like episodes)
MERFF (myoclonic epilepsy with ragged-red fibers)
Metachromatic leukodystrophy
Multiple sulfatase deficiency
Neonatal adrenoleukodystrophy
Refsum disease

CHERRY RED MACULAR SPOTS

Galactosialidosis
GM_1 gangliosidosis
Mucolipidosis I
Multiple sulfatase deficiency
Niemann-Pick disease
Sandhoff disease
Sialidosis
Tay-Sachs disease

CHOLESTATIC JAUNDICE

Alagille syndrome
α_1-Antitrypsin deficiency
Bile acid synthesis defects
Byler disease (progressive familial intrahepatic cholestasis [PFIC1, BRIC1])
PFIC2 (bile salt excretory pump BSEP)
PFIC 3 (MDR3)
Citrin deficiency
Cystic fibrosis
Dubin-Johnson syndrome
Fructose bisphosphate aldolase (B) deficiency (HNF-1β)
Hepatic nuclear factor 1β gene mutations
Hepatorenal tyrosinemia
LCHAD deficiency
Mevalonic aciduria
Mucolipidosis type II
Niemann-Pick disease
Niemann-Pick type C disease
Peroxisomal biogenesis disorders
Rotor syndrome
Tyrosinemia, hepatorenal

CHONDRODYSPLASIA PHENOTYPES

Conradi-Hünermann syndrome
Mucolipidosis II, I-cell disease
Peroxisomal disorders
Warfarin embryopathy

CHRONIC PANCREATITIS

Hereditary (dominant) (with or without lysinuria (cystinuria)): with or without pancreatic lithiasis or portal vein thrombosis
With hyperparathyroidism in multiple endocrine adenomatosis syndrome
MELAS
Mitochondrial disorders (e.g. Pearson, Kearns–Sayre and MELAS syndromes)
Organic acidemia
Pearson syndrome
Regional enteritis (Crohn)
Trauma – pseudocyst

CIRRHOSIS OF THE LIVER

α 1-Antitrypsin deficiency
Cholesterylester storage disease
Citrin deficiency (citrullinemia)
Cystic fibrosis
Congenital disorders of glycosylation
Defects of bile acid synthesis
Electron transport chain disorders
Fructose intolerance
Galactosemia
Gaucher disease
Glycogenosis types IV and IX
Hemochromatosis
Hepatorenal tyrosinemia
Hypermethioninemia
Mitochondrial DNA depletion
Niemann-Pick type C
Peroxisomal disorders
Phosphoenolpyruvate carboxykinase deficiency
Progressive intrahepatic cholestasis
Pyruvate kinase deficiency
Thalassemia
Transaldolase deficiency
Wilson disease
Wolman disease

CORNEAL OPACITY

Cystinosis
Fabry
Fish eye disease (LCAT deficiency)
Galactosialidosis
GM_1 gangliosidosis
Hurler disease (MPS I)
I-cell disease
Mannosidosis
Mucolipidosis III
Multiple sulfatase deficiency

CORPUS CALLOSUM AGENESIS

Adrenocorticotrophic hormone (ACTH) deficiency
Aicardi syndrome
Mitochondrial disorders (especially pyruvate dehydrogenase deficiency)
Nonketotic hyperglycinemia
Peroxisomal disorders
Pyruvate dehydrogenase complex deficiency
VICI syndrome

CREATINE KINASE – ELEVATED

Aldolase A deficiency
Carnitine palmitoyl transferase II deficiency
COL4A1
Congenital disorders of glycosylation (CDG)
Defects of glycogenolysis
Disorders of fatty acid oxidation
Glycogenosis – III
Glycogenosis – V – McArdle
Glycogenosis – phosphofructokinase
D-2-Hydroxyglutaric aciduria
3-Oxothiolase deficiency
Mevalonic aciduria
Mitochondrial disorders
Myoadenylate deaminase deficiency

DEAFNESS, SENSINEURAL

α-Mannosidosis
Biotinidase deficiency
Canavan disease
Fabry disease
MEDNIK syndrome
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
Mitochondrial disorders (including Pearson, Kearns–Sayre, MELAS, MERRF and MIDD syndromes, succinyl-CoA synthase deficiency, BCS1L mutations presenting as complex III deficiency or Björnstad syndrome)
Peroxisomal disorders
PRPP synthetase abnormality
Refsum disease
SLC33A1 deficiency (MIM 614482)
VICI syndrome

DERMATOSIS

Acrodermatitic enteropathica
Biotinidase deficiency
Holocarboxylase synthetase deficiency

DIABETES MELLITUS – ERRONEOUS DIAGNOSIS

Congenital disorders of glycosylation
Isovaleric acidemia
Methylmalonic acidemia
3-Oxothiolase deficiency
Propionic acidemia

DIARRHEA

Abetalipoproteinemia
Congenital chloride diarrhea
Electron transport disorders
Enterokinase deficiency
Glucose galactose malabsorption
Johansson-Blizzard syndrome
Lactase deficiency
Lysinuric protein intolerance
Pearson syndrome
Schwachman syndrome
Sucrase deficiency
Wolman disease

DISORDERS OF FOLATE METABOLISM OR TRANSPORT

Cerebral folate transport deficiency
Dihydrofolate reductase deficiency (DHFR)
Glutamate formiminotransferase deficiency
Hereditary folate malabsorption
Methionine synthase reductase deficiency (CbIE)
Methionine synthetase deficiency (CbIG)
Methylene tetrahydrofolate reductase (MTHFR) deficiency

DRUGS OR OTHER TRIGGERS OF SYMPTOMS IN METABOLIC DISEASES

Infection
Neuroleptics (Wilson disease, HPRT deficiency)
Porphyrogenic drugs (imipramine, meprobamate), alcohol
Surgery
Valproate

DYSOSTOSIS MULTIPLEX

Galactosialidosis
Generalized GM_1 gangliosidosis
Hurler, Hurler-Scheie disease
Hunter disease
Maroteaux-Lamy disease
Mucolipidosis II, I-cell disease

Mucolipidosis III
Multiple sulfatase deficiency
Sanfilippo disease
Sly disease

ECTOPIA LENTIS (DISLOCATION OF THE LENS)

Homocystinuria
Hyperlysinemia
Marfan syndrome
Molybdenum cofactor deficiency
Sulfite oxidase deficiency
Weiss-Marchesani syndrome

EEG BURST SUPPRESSION PATTERN

Anesthesia – deep stages
Anoxia, cerebral hypoperfusion
Drug overdose (e.g. phenobarbital)
Molybdenum cofactor deficiency
Nonketotic hyperglycinemia
Organic acidemia (neonatal encephalopathy-propionic acidemia)
Sulfite oxidase deficiency

EXERCISE INTOLERANCE

Defects of glycogenolysis
Disorders of fatty acid oxidation
3-Oxothiolase deficiency
Mitochondrial disorders
Myoadenylate deaminase deficiency

FAILURE TO THRIVE

α_1-Antitrypsin deficiency
Cobalamin C disease
Congenital disorders of glycosylation
Glycogenosis II
Hepatorenal tyrosinemia
Lysinuric protein intolerance
Methylmalonic acidemia
Mevalonic aciduria
Niemann-Pick disease
Pearson syndrome
Propionic acidemia
Wolman disease

FEVER SYNDROMES

Familial mediterranean fever
Hyperimmunoglobulin D syndrome (mevalonic aciduria)
Muckle-Wells syndrome (neonatal onset multisystem inflammatory disease syndrome)
Tumor necrosis factor receptor-associated periodic syndrome

GLYCOSURIA

Cystinosis
Diabetes mellitus
Hepatorenal tyrosinemia
Fanconi-Bickel syndrome – GLUT-2 mutations
Glycogen synthase deficiency
Pearson syndrome
Renal Fanconi syndrome
Wilson disease

HAIR ABNORMALITIES

Argininosuccinic aciduria
Björnstad syndrome (complex 3)
Homocystinuria (Fine, brittle hair)
Infantile sialic acid storage disease (fair hair)
Kinky hair, photosensitivity, and impaired mental development
Menkes disease (pili torti, trichorrhexis nodosa, monilethrix)
Pili torti: isolated, MIM 261900
Pili torti with deafness or with dental enamel hypoplasia MIM 262000 (Bjornstad syndrome)
Trichothiodystrophy: trichorrhexis nodosa, ichthyosis, and neurological abnormalities (Pollit syndrome) MIM 27550
VICI syndrome (hypopigmented hair)

HDL (LIPOPROTEIN) LOW

Lecithin cholesterol acyltransferase (LCAT) deficiency (fish eye disease)
Tangier disease
Hypoalphalipoproteinemia

HEMOLYTIC ANEMIA

Defects of glycolysis
5-Oxoprolinuria
Purine and pyrimidine disorders
Wilson disease

HEMOPHAGOCYTOSIS (ERYTHROPHAGOCYTOSIS)

Carnitine palmitoyl transferase I
Familial hemophagocytic lymphocytic histocytosis (perforin deficiency, PRF1)

Gaucher disease
Hemochromatosis
Lysinuric protein intolerance
Niemann-Pick disease
Propionic acidemia
Wolman disease

HEPATIC CARCINOMA

α_1-Antitrypsin deficiency
Galactosemia
Gaucher disease
Glycogen storage disease types I and IV
Hemochromatosis
Hepatorenal tyrosinemia
Progressive intrahepatic cholestasis
Thalassemia
Wilson disease

HEPATIC FAILURE – ACUTE

α_1-Antitrypsin deficiency
Bile acid disorders
MPI-CDG, formally CDG Ib
Citrullinemia
Fatty acid oxidation disorders
Galactosemia
Hepatorenal tyrosinemia
Hereditary fructose intolerance
LARS (IFLS1)
LCHAD/mTP-Mangel
Mitochondrial disorders (especially mitochondrial DNA depletion syndromes caused by POLG, DGUOK and MPV17 mutations; also TRMU mutations)
NBAS
Neonatal hemochromatosis
Niemann-Pick types B and C
Transaldolase deficiency
Wilson disease
Wolcott–Rallison syndrome

HYDROPS FETALIS

Carnitine transporter deficiency
Congenital disorders of glycosylation
Farber disease (disseminated lipogranulomatosis)
Galactosialidosis
Glycogenosis type IV
GM_1 gangliosidosis
Gaucher disease
Infantile free sialic acid storage disease (ISSD)
Mucolipidosis II – I-cell disease
Neonatal hemochromatosis
Niemann-Pick disease
Niemann-Pick disease type C
Pearson syndrome (anemia)
Sialidosis
Sly disease (β-glucuronidase deficiency)
Wolman disease

3-HYDROXYGLUTARIC ACIDURIA

Glutaryl CoA dehydrogenase deficiency
Short chain hydroxyacyl CoA dehydrogenase deficiency
Carnitine palmitoyltransferase I deficiency

HYPERAMMONEMIA

N-Acetylglutamate synthetase deficiency
α_1-Antitrypsin deficiency
Argininemia
Argininosuccinic aciduria
Carbamoyl phosphate synthetase deficiency
Carnitine palmitoyl transferase-II deficiency
Chemotherapy-induced hyperammonemia
Citrullinemia
Fatty acid oxidation disorders
HHH syndrome
HMG CoA lyase deficiency
Hyperthermia, malignant
Isovaleric acidemia
Lysinuric protein intolerance
MCAD deficiency
Methylmalonic acidemia
Mitochondrial carbonic anhydrase VA deficiency
Multiple carboxylase deficiency
Ornithine transcarbamylase deficiency
Δ1-pyrroline-5-synthase deficiency
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex deficiency
Propionic acidemia
Transient hyperammonemia of the newborn
Urinary tract infection – urea-splitting bacteria
Valproate
Wilson disease

HYPERCALCEMIA

Acute renal failure
Endocrinopathies
  Thyrotoxicosis
  Adrenal insufficiency
  Pheochromocytoma
  Vasoactive intestinal peptide-producing tumor (VIPoma)
Immobilization-associated
Malignancy-associated hypercalcemia

Milk-alkali syndrome
Primary hyperparathyroidism and variants
  Sporadic primary hyperparathyroidism
  Familial primary hyperparathyroidism
    Associated with multiple endocrine neoplasia type 1 (MEN-1)
    Associated with multiple endocrine neoplasia type 2 (MEN-2)
  Familial benign hypocalciuric hypercalcemia
  Tertiary hyperparathyroidism
    In chronic renal failure
    After renal transplantation
    Associated with lithium therapy
Sarcoidosis and other granulomatous diseases
Vitamin A intoxication
Vitamin D intoxication

HYPERTYROSINEMIA

Deficiency of 4-hydroxyphenylpyruvate dioxygenase
Drug – toxin
Hepatic infection
Hepatorenal tyrosinemia
Hyperthyroidism
Oculocutaneous tyrosinemia
Postprandial
Scurvy
Transient tyrosinemia of the newborn
Treatment with NTBC
Tyrosinemia type III

HYPOKETOTIC HYPOGLYCEMIA

Adenosine kinase deficiency
Carnitine transporter deficiency
CPT I deficiency
3-α-Hydroxyacyl- CoA dehydrogenase deficiency (HADH deficiency, formerly SCHAD)
HMG CoA lyase deficiency
LCAD
LCHAD
MCAD
Monocarboxylate transporter 1 deficiency
VLCAD

HYPOPHOSPHATEMIA

Fanconi syndrome
Hyperparathyroidism
MELAS
Pearson
X-linked hypophosphatemic rickets

HYPOURICEMIA

Fanconi syndrome, cystinosis, any proximal renal tubular dysfunction
Isolated renal tubular defect (Dalmatian dog model)
Molybdenum cofactor deficiency
Phosphoribosyl pyrophosphate synthetase deficiency
Purine nucleoside phosphorylase deficiency
Wilson disease
Xanthine oxidase deficiency

ICHTHYOSIS

ARC syndrome
CEDNIK syndrome
CHILD syndrome (congenital hemidysplasia, ichthyosis, and limb defects)
COG5-CDG (CDG IIi)
Conradi–Huenermann syndrome
Gaucher disease
Krabbe disease
MPDU1-CDG (CDG If)
Multiple sulfatase deficiency
Refsum disease
Rhizomelic chondrodysplasia punctata type I
Serine deficiency syndromes
Sjogren-Larsson syndrome
X-linked ichthyosis – steroid sulfatase deficiency

ICHTHYOSIS AND RETINAL DISEASE

Refsum syndrome
Sjogren-Larsson syndrome

INVERTED NIPPLES

Biopterin synthesis disorders
Citrullinemia
Congenital disorders of glycosylation
Glycogenosis 1b
Hyperphenylalaninemia
Isolated – dominant (MIM 163610)
Isovaleric acidemia
Menkes disease
Methylmalonic acidemia
Molybdenum cofactor deficiency
Niemann-Pick type C
Propionic acidemia
Pyruvate carboxylase deficiency
SCAD deficiency
VLCAD deficiency
Weaver syndrome

ISOLATED DEFICIENCY OF SPEECH AS PRESENTATION IN METABOLIC DISEASE

D-Glyceric aciduria
Electron transport chain disorders
3-Methylglutaconyl CoA hydratase

LACTIC ACIDEMIA

Electron transport chain disorders
Fatty acid oxidation disorders (long-chain)
Krebs cycle defects
Lues, congenital
MELAS
MERRF
Organic acidemias, e.g. propionic acidemia
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase deficiency

LEIGH SYNDROME

Biotinidase deficiency
ECHS1 deficiency
Electron transport chain abnormalities
Ethylmalonic Encephalopathy
Fumarase deficiency
HIBCH deficiency
MEGDEL (3-methylglutaconic aciduria type VI, MGCA6)
3-Methylglutaconic aciduria
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex deficiency
Sulfite oxidase deficiency

LEUKOPENIA WITH OR WITHOUT THROMBOPENIA AND ANEMIA

Abnormalities of folate metabolism
Isovaleric acidemia
Johansson-Blizzard syndrome
Methylmalonic acidemias
3-Oxothiolase deficiency
Pearson syndrome
Propionic acidemia
Schwachman syndrome
Transcobalamin II deficiency
Transaldolase deficiency

MACROCEPHALY

Alexander disease
Bannayan-Ruvalcaba-Riley syndrome
Canavan disease
Glutaric aciduria type I
Hurler disease
4-Hydroxybutyric aciduria
3-Hydroxy-3-methylglutaric aciduria
L-2-Hydroxyglutaric aciduria
Krabbe disease
Mannosidosis
Multiple acyl CoA dehydrogenase deficiency
Multiple sulfatase deficiency
Neonatal adrenoleukodystrophy
Pyruvate carboxylase deficiency
Tay-Sachs disease

MEGALOBLASTIC ANEMIA

B_{12} deficiency – vegan or breastfed infant of vegan mother or mother with pernicious anemia
Cobalamin metabolic errors-methylmalonic acidemia and homocystinuria-Cbl C and D
Folate metabolism, abnormalities of CblF cobalamin lysosomal transporter deficiency
Dietary folate deficiency
Folate malabsorption – hereditary – protein coupled folate transport (PCFT) deficiency
Intestinal B_{12} transport deficiency – Immerslund-Grasbeck-Cubilin deficiency
Methylmalonic acidemia-homocystinuria-Cbl C+D
Mevalonic aciduria
Orotic aciduria
Pearson syndrome
Pernicious anemia – intrinsic factor deficiency
Thiamine responsive megaloblastic anemia (OMIM 249270) (thiamine transporter [TRMA] mutation)
Transcobalamin II deficiency

METABOLIC ACIDOSIS AND KETOSIS

Fabry disease
Familial hypocholesterolemia
Homocystinuria
Isovaleric acidemia
Menkes disease
Methylcrotonyl CoA carboxylase deficiency
Methylmalonic acidemia
Multiple carboxylase deficiency
Propionic acidemia

METHYLMALONIC ACIDURIA

B_{12} deficiency, pernicious anemia, including autoimmune
Cobalamin A
Cobalamin C, D

Imerslund-Gräsbeck – cobalamin enterocyte malabsorption
Mut^0, Mut$^-$
Succinyl CoA synthase deficiency
Transcobalamin II deficiency

MONGOLIAN SPOT – EXTENSIVE

GM_1 gangliosidosis
Hurler syndrome
Hunter syndrome
Mannosidosis
Niemann-Pick disease

MYOCARDIAL INFARCTION–CEREBRAL VASCULAR DISEASE

Fabry disease
Familial hypercholesterolemia
Homocystinuria
Menkes disease
Oxothiolase deficiency
Propionic acidemia
SCHAD deficiency

NEONATAL HEPATIC PRESENTATIONS IN METABOLIC DISEASES

Adenosine kinase deficiency
α_1-Antitrypsin deficiency
Cystic fibrosis
Galactosemia
Hemochromatosis
Hepatorenal tyrosinemia
Long-chain hydroxy-acyl CoA dehydrogenase deficiency
Mitochondrial DNA depletion syndromes
MPI-CDG, formally CDG Ib
NBAS
Niemann-Pick type C disease
Wilson disease
Wolman disease
Sly disease
Transaldolase deficiency

NEUTROPENIA

Isovaleric acidemia
Methylmalonic aciduria
Propionic acidemia
Cartilage hair hypoplasia
Cyclic neutropenia
Leukemia
Oxothiolase deficiency
Splenic neutropenia
Drug-induced agranulocytosis
Idiopathic congenital neutropenias

ODD OR UNUSUAL ODOR

Dimethylglycinuria
Glutaric aciduria type II
Hepatorenal tyrosinemia
Isovaleric acidemia
Maple syrup urine disease
Phenylketonuria
Trimethylaminuria
Treatment of urea cycle disorder with phenylacetate

OPTIC ATROPHY

ADP-ribosyl protein lyase deficiency
Adrenoleukodystrophy (ALD)
Biotinidase deficiency
Canavan disease
GM_1 gangliosidosis
Homocystinuria
Krabbe disease
Menkes disease
MERRF
Metachromatic leukodystrophy
3-Methylglutaconic aciduria, type III (Costeff)
Mevalonic aciduria
Mitochondrial energy metabolism, defects in – including Leber hereditary optic neuropathy (LHON)
Multiple sulfatase deficiency
NARP
Neonatal adrenoleukodystrophy
Propionic acidemia
Sandhoff disease
Tay-Sachs disease

OROTIC ACIDURIA

UMP synthase deficiency (hereditary orotic aciduria)
Urea cycle defects – ornithine transcarbamylase deficiency, citrullinemia, argininosuccinic aciduria, arginemia
Purine nucleoside phosphorylase (PNP) deficiency
Phosphoribosylpyrophosphate (PRPP) synthetase deficiency

OSTEOPOROSIS AND FRACTURES

Adenosine deaminase deficiency
Gaucher disease
Glycogenosis I
Homocystinuria

I-cell disease
Infantile Refsum disease
Lysinuric protein intolerance
Menkes disease
Methylmalonic acidemia
Propionic acidemia

PAIN AND ELEVATED ERYTHROCYTE SEDIMENTATION RATE

Fabry disease
Familial hypercholesterolemia
Gaucher disease
Mevalonic aciduria

PANCREATITIS

Carnitine palmitoyl transferase I deficiency
Carnitine palmitoyl transferase II deficiency
Cytochrome c oxidase deficiency
Glycogenosis type I
Glycogenosis 1 plus apoE2 type III hypertriglyceridemia
Hereditary dominant, with or without lysinuria;
  with or without pancreatic lithiasis or portal vein thrombosis
Homocystinuria
Hydroxymethylglutaryl CoA lyase deficiency
Hyperlipoproteinemia type IV
Isovaleric acidemia
Lesch-Nyhan disease
Lipoprotein lipase deficiency, also type IV
Lysinuric protein intolerance
Maple syrup urine disease
MELAS
Methylmalonic acidemia
Ornithine transcarbamylase deficiency
Pearson syndrome
Propionic acidemia
Regional enteritis (Crohn)
Trauma – pseudocyst
With hyperparathyroidism in multiple endocrine adenomatosus syndrome

PARALYSIS OF UPWARD GAZE

Leigh; Kearns-Sayre syndromes
Niemann-Pick type C
Peripheral neuropathy

PHOTOPHOBIA

Cystinosis
Oculocutaneous tyrosinemia

POLYCYSTIC KIDNEYS

Carnitine palmitoyl transferase II (CPT-II) deficiency
Congenital disorders of glycosylation
Multiple acyl CoA dehydrogenase deficiency, glutaric aciduria type II (GA II)
Zellweger syndrome

POSITIVE NEWBORN SCREEN OF NORMAL INFANT; MOTHER IS DEFICIENT

Carnitine transporter deficiency
Holocarboxylase synthetase deficiency
Methylcrotonyl-CoA carboxylase deficiency
Very long chain acylCoA dehydrogenase deficiency
Vitamin B12 deficiency

PSYCHIATRIC PRESENTATIONS

Acute and recurrent confusion/psychosis
  Homocystine remethylation defects (MTHFR deficiency, Cblc)
  Porphyrias
  Urea cycle, especially OTC deficiency
Chronic psychotic disease
  Adrenoleukodystrophy
  Homocystinurias
  Lysosomal diseases, metachromatic leukodystrophy, GM_2 gangliosidosis
  Wilson disease
Late onset behavioral personality changes
  Cerebral tendenous xanthomatosis
  Ceroid lipofuscinosis
  Creatine transporter effect
  Homocystinurias
  4-Hydroxybutryic aciduria
  Mannosidosis, α and β
  Monoamine oxidase deficiency
  Niemann-Pick type C
  Nonketotic hyperglycinemia (late onset)
  Sanfilippo disease

PSYCHOTIC BEHAVIOR

Carbamoyl phosphate synthetase deficiency
Cbl disease
Ceroid lipofuscinosis
Citrullinemia
Hartnup disease
Homocystinuria
Hurler-Scheie, Scheie disease
Krabbe disease

Lysinuric protein
Maple syrup urine disease
$MeFH_4$ reductase deficiency
Metachromatic leukodystrophy
Mitochondrial disease (MELAS)
Neuronal ceroid lipofuscinoses
Niemann-Pick type C disease
Ornithine transcarbamylase deficiency
Porphyria
Sanfilippo disease
Tay-Sachs, Sandhoff-late onset
Wilson disease

PTOSIS

Congenital myasthenic syndromes
Dopamine deficiency syndromes
Kearn-Sayre syndrome
MNGIE (mitochondrial neurogastrointestinal encephalomyelopathy)

PYLORIC STENOSIS, VOMITING AND ERRONEOUS DIAGNOSIS OF

Ethylmalonic-adipic aciduria
Galactosemia
HMG CoA lyase deficiency
4-Hydroxybutyric aciduria
D-2-Hydroxyglutaric aciduria
Isovaleric acidemia
Methylmalonic acidemia
Molybdenum cofactor deficiency
3-Oxothiolase deficiency
Phenylketonuria
Propionic acidemia

RAGGED RED FIBERS

Menkes disease
Mitochondrial DNA mutations

RED URINE

Beets
Congenital erythropoietic porphyria
Drugs: ibuprofen, nitrofurantoin, phenolphthalein, pyridium, rifampicin
Hematuria
Hemoglobinuria
Myoglobinuria
Red diaper syndrome (*Serratia marcescens*)
Red dyes (Monday morning disorder, rhodamine B)

RENAL CALCULI

APRT (adenosine phosphoribosyltransferase) deficiency
Cystinuria
HPRT deficiency–Lesch-Nyhan disease
Oxaluria
PRPP synthetase abnormalities
Wilson disease
Xanthine oxidase deficiency

RENAL CYSTS

Carnitine palmitoyl transferase deficiency
Congenital disorders of glycosylation

RENAL FANCONI SYNDROME

Cystinosis
Galactosemia
Glycogenosis I and III
Hepatorenal tyrosinemia
Lowe syndrome
Lysinuric protein intolerance
Mitochondrial disorders (especially Pearson and Kearns-Sayre syndromes, BCS1L mutations)
Primary Fanconi syndrome
Wilson disease

RENAL TUBULAR ACIDOSIS (RTA)

Cystinosis
Fanconi syndrome
Galactosemia
Hepatorenal tyrosinemia
Mitochondrial electron transport defect
Osteopetrosis and RTA
Topamax

RETINITIS PIGMENTOSA

Abetalipoproteinemia
Congenital disorders of glycosylation
Ceroid lipofuscinosis
Hunter disease
Kearns-Sayre syndrome
LCHAD deficiency
Mevalonic aciduria
NARP
Peroxisomal biosynthesis disorders
Primary retinitis pigmentosa
Refsum disease
Sjogren-Larsson syndrome (fatty alcohol oxidoreductase deficiency)

REYE SYNDROME PRESENTATION

Fatty acid oxidation, disorders of
Gluconeogenesis, abnormalities of
Electron transport chain abnormalities
Fructose intolerance
Infantile Liver failure syndrome type I (IFLS1, LARS)
NBAS
Organic acidemias
Urea cycle, disorders of

REYNAUD SYNDROME

Fabry disease

RHABDOMYOLYSIS

Aldolase A (fructose bisphosphate) deficiency
Disorders of fatty acid oxidation – LCHAD, VLCAD, CPTII
Drugs – methylenedioxymethylamphetamine (MDMA)
Glutaric acidemia I
Glycogenosis
  V McArdle – myophosphorylase
  VII Tarui – phosphofructokinase
Glycolysis
  phosphoglycerate kinase
  phosphoglyceromutase
Infection – myositis
Ischemic injury
LPIN1 mutations
Mitochondrial DNA deletions (multiple)
Mitochondrial point mutations (MELAS)
Oxphos defects – complex I, complex II
Quail ingestion – coturnism
Toxin-tetanus, snake venom, alcohol, cocaine, bee venom

SCOLIOSIS

CDG
Ehlers-Danlos syndromes
Homocystinuria
Marfan syndrome
Pyruvate dehydrogenase complex deficiency

SELF-INJURIOUS BEHAVIOR

De Lange syndrome
Familial dysautonomia
Hepatorenal tyrosinemia
Lesch-Nyhan disease
Phenylketonuria
Smith-Magenis syndrome
Sensory neuropathy

SPASTIC PARAPARESIS

Argininemia
Biotinidase deficiency
HHH syndrome
Metachromatic leukodystrophy
Pyroglutamic aciduria
Sjögren-Larsson syndrome

STROKE-LIKE EPISODES

Carbamyl phosphate synthetase deficiency
Chediak-Higashi syndrome
Citrullinemia
Congenital disorders of glycosylation
Cystinosis
Ethylmalonic aciduria
Fabry disease
Familial hypercholesterolemia
Glutaric aciduria type I
Homocystinuria
Hydroxymethylglutaryl-CoA lyase deficiency
Isovaleric acidemia
MELAS and other mitochondrial disorders
Menkes disease
Methylcrotonyl CoA carboxylase deficiency
Methylmalonic acidemia
3-Methylene FH4 reductase deficiency
Multiple acyl CoA dehydrogenase deficiency
Ornithine transcarbamylase deficiency and other urea cycle disorders
Propionic acidemia
Phosphoglycerate kinase deficiency
Phosphorylase kinase deficiency
Progeria
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase deficiency
Respiratory chain disorder
Purine nucleoside phosphorylase deficiency
Sulfite oxidase deficiency

SUBDURAL EFFUSIONS

Glutaric aciduria (I)
D-2-Hydroxyglutaric aciduria
Menkes disease
Pyruvate carboxylase deficiency

TEETH COLORED

Amelogenesis imperfecta (dense white, lyonized)
Dentinogenesis imperfecta (blue gray or brown)
Fluorosis
Hyperbilirubinemia (green)

Hypothyroidism, congenital
Kohlschutter-Tonz syndrome
Porphyria, congenital erythropoietic (red, fluorescent)
Tetracycline (brown, fluorescent)
Tyrosinemia, oculocutaneous

VISUAL HALLUCINATIONS

Cobalamin C disease
Maltase (acid) deficiency
α-Mannosidosis
Methylene tetrahydrofolate reductase (MTHFR) deficiency
Niemann-Pick disease type C
Propionic acidemia
Tay-Sachs disease (late onset)
Urea cycle defects

XANTHOMAS

Cerebrotendinous xanthomatosis
Familial hypercholesterolemia
Lipoprotein lipase deficiency
Niemann-Pick disease
Sitosterolemia

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