

7.1 Introduction

The disorders of valine and isoleucine metabolism comprise quite distinct diseases.

Biotinidase deficiency [1, 2] is a form of multiple carboxylase deficiency in which the failure to cleave biocytin to yield biotin and lysine leads to biotin deficiency, producing deficient activity of each of the carboxylases, especially the mitochondrial enzymes propionyl-CoA carboxylase, 3-methylcrotonyl CoA carboxylase and pyruvate carboxylase.

Clinical presentation may be that of a typical organic acidemia with life-threatening ketoacidosis early in life, or the onset may be more indolent with symptoms linked to the skin, hair or the nervous system. Some patients have presented as acrodermatitis enteropathica. Others have had immunodeficiency. In some seizures have been the only clinical manifestation. Later onset patients have had spastic diplegia or atrophy of the optic or auditory nerves.

Analysis of organic acids of the urine reveals the picture of multiple carboxylase deficiency characterized by pronounced excretion of lactate and 3-hydroxyisovalerate, 3-methylcrotonylglycine, 3-hydroxypropionate and methylcitrate.

Holocarboxylase synthetase deficiency [3, 4] is the classic infantile form of multiple carboxylase deficiency. Untreated it is uniformly fatal, while early diagnosis and treatment with biotin usually lead to the disappearance of all of the manifestations of the disease. Life-threatening illness is associated with massive ketosis and metabolic acidosis. A bright red cutaneous eruption may cover the body, and there is alopecia totalis. Immune function, both T and B cell, may be defective.

Defective activity of holocarboxylase synthetase renders it impossible to activate carboxylase enzymes. This leads to defective activity of all of the carboxylases. Organic acid analysis reveals a pattern resembling biotinidase deficiency.

Propionic acidemia [5, 6] is a result of defective activity of propionyl-CoA carboxylase. The clinical presentation includes life-threatening episodes of ketosis and acidosis. A small subpopulation has a neurologic pre-

sentation without the heralding ketoacidotic attacks. The hematological manifestations include neutropenia and, in infancy, thrombocytopenia and anemia.

Acute episodes are characterized by massive ketonuria. Amino acid analysis reveals large amounts of glycine in plasma and urine. The diagnosis is best made by organic acid analysis of the urine. The key compounds are methylcitrate and 3-hydroxypropionate. GCMS analysis for methylcitrate provides for a rapid and specific approach to prenatal diagnosis.

3-Oxothiolase deficiency [7] is associated with recurrent episodes of vomiting, ketosis and acidosis. Some patients have had hypoglycemia. Neutropenia and thrombocytopenia have been observed in infancy. Elevated concentrations of glycine have been observed in the blood and urine of some, but not all, patients. The key metabolites are 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid and tiglylglycine. These compounds increase in amounts in the urine following loading with isoleucine. The fundamental defect is in the activity of 2-methylacetoacetyl-CoA thiolase.

Methylmalonate semialdehyde dehydrogenase deficiency [8] has been described in a single patient. This patient, a boy, came to attention because of an elevated concentration of methionine on routine neonatal screening. The value exceeded 1000 $\mu\text{mol/l}$. By 4 years of age he had developed normally. A valine load was followed by an increase in 3-hydroxyisobutyric acid excretion. Incubation of fibroblasts from the patient with 2- ^{14}C -valine or β -[1- ^{14}C]-alanine led to no production of $^{14}\text{CO}_2$ from valine and very little from β -alanine in contrast to control cells.

Examination of plasma and urine revealed elevated quantities of β -alanine, 3-hydroxypropionic acid, (R)- and (S)-3-aminoisobutyric acid, (R)- and (S)-3-hydroxyisobutyric acid and (S)-2-hydroxymethylbutyric acid. Direct enzymatic assay of methylmalonate semialdehyde dehydrogenase is unavailable revealed homozygosity for DNA analysis 1336 G>A transversion which substituted an arginine for a highly conserved glycine at amino acid residue 446.

3-Hydroxyisobutyric aciduria in which methylmalonate semialdehyde activity is normal [9, 10] is another inborn error of valine metabolism. One patient [9] had a typical organic acidemia phenotype with recurrent episodes of vomiting, ketosis and acidosis, and dehydration. He had lactic acidemia and hyperalaninemia. Organic acid analysis of the urine revealed large amounts of 3-hydroxyisobutyric acid and 2-ethyl-3-hydroxypropionic acid. Loading with valine reproduced the clinical illness with vomiting, sweating, ketosis and acidosis. Urinary excretion of lactic acid, 3-hydroxyisobutyric acid and 3-aminoisobutyrate rose markedly. Cultured fibroblasts were defective in the conversion of [^{14}C]valine and [^{14}C] β -alanine to $^{14}\text{CO}_2$ [10]. These observations, and the excretion of 2-ethyl-3-hydroxypropionate, suggest a defect in a semialdehyde dehydrogenase active on methylmalonic semialdehyde, malonic semialdehyde and ethylmalonic semialdehyde. A sec-

and patient with 3-hydroxyisobutyric acidemia had malformations, massive acidosis and hypotonia.

The evolution of tandem mass spectrometry (MS) for the analysis of acylcarnitines of blood and fibroblasts has been critical in the identification of previously unrecognized inborn errors of L-isoleucine degradation, 2-methylbutyryl-CoA dehydrogenase [11, 12] and 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiencies [13].

An infant with 2-methylbutyryl CoA dehydrogenase deficiency [11] was admitted at 3 days of life with a life-threatening episode characterized by hypoglycemia, dehydration, lethargy and hypothermia. Acidosis was mild without ketosis. MRI revealed increased signal in the lentiform nuclei. By 12 months of age he was not achieving milestones and carried a diagnosis of athetoid cerebral palsy. 2-Methylbutyryl carnitine was found in blood, and 2-methylbutyrylglycine in urine. The conversion of ^{14}C -isoleucine to $^{14}\text{CO}_2$ 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 2-methylbutyryl CoA dehydrogenase. Mutation analysis revealed a 778 C>T substitution in the coding region which led to the substitution of a phenylalanine for leucine at amino acid 222. A second pregnancy in this family produced an affected sister who appeared healthy at report.

Another patient [12] was a 3-year-old product of a consanguineous mating who had hypotonia and retarded motor development. MRI was normal. He had 2-methylbutyrylglycinuria, but a normal acylcarnitine profile. His asymptomatic mother also excreted 2-methylbutyrylglycine. The activity of 2-methylbutyryl-CoA dehydrogenase in fibroblasts was 10% of control. Mutation analysis revealed homozygosity for a G>A 1228 transition in the patient and his mother.

2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency was reported [13] in a 2-year-old who had progressive loss of motor and cognitive development. By 2 years he was severely retarded, hypotonic and displayed choreathetoid movements and seizures. He manifested tachypnea metabolic acidosis and impressive hypoglycemia on the 2nd day of life, when he was found to have lactic acidemia, hyperammonemia and ketonuria. Urinary organic acid analysis at 2 months revealed elevated tiglylglycine and 2-methyl-3-hydroxybutyrate, and he was thought to have 3-oxothiolase deficiency. Tandem mass spectrometry revealed intermittent elevations of C5:1(tiglyl)-carnitine and C5-OH-(2-methyl-3-hydroxybutyryl) carnitine. Activity of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase in fibroblasts was deficient.

The methylmalonic acidemias [14, 15] are a family of disorders in the metabolism of branched-chain amino acids in which the activity of methylmalonyl-CoA mutase is defective. They may be divided into mutase apoenzyme defects and defects in cofactor synthesis or cobalamin metabolism.

The former do not respond to vitamin B₁₂ while the latter do. Among the defects in cobalamin metabolism, of four complementation groups two (cblA and B) represent defects in the synthesis of deoxyadenosylcobalamin while the other two (cblC and D) represent a combined abnormality in which there is also defective synthesis of methylcobalamin, the cofactor for methionine synthesis [16].

The clinical manifestations of methylmalonic acidemia are predominantly those of overwhelming illness very early in life. A typical episode is ushered in with ketonuria and vomiting and progresses to dehydration, acidosis, lethargy and coma. In the absence of intensive resuscitation, and sometimes despite it, the outcome is fatal.

Organic acid analysis of the urine reveals large amounts of methylmalonic acid.

Patients with methylmalonic aciduria and homocystinuria represent defective metabolism of cobalamin to the two cofactors methylcobalamin and deoxyadenosylcobalamin. Hence the activity of methionine synthase, and that of methylmalonyl-CoA mutase are defective [16]. The patients fall into two distinct complementation groups, designated cblC or cblD. An additional group of patients – designated group cblF – have defective transport of free cobalamin out of lysosomes [17].

The clinical manifestations of the cblC disease, which is the most common, are those of megaloblastic anemia and failure to thrive. Death may occur within the first 6 months of life. Some patients have seizures. Patients with onset later than the early months of life have had predominantly neurologic presentations, with spasticity, myelopathy or dementia. Hematologic examination reveals a pattern of pernicious anemia.

Only two patients have been reported with cblD disease, brothers, neither of whom was anemic. One was mentally retarded, psychotic and had abnormalities of spinal cord and cerebellar function with ataxia. His 2-year-old affected brother was well.

The only patient reported with cblF disease [17] presented at 2 weeks of age with stomatitis, hypotonia and seizures. By 8 months she was developmentally delayed. There were no hematologic abnormalities.

A single patient with multiple congenital malformations was reported with isolated 3-hydroxyisobutyryl-CoA deacylase deficiency [18]. Intact cell oxidation of [¹⁴C]-valine revealed decreased pathway function, and direct analysis in extracts of fibroblasts derived from the patient revealed deficient deacylase activity. The urine contained increased amounts of unusual sulfhydryl adducts, S-(2-carboxypropyl)cysteine and S-(2-carboxypropyl)cysteamine, believed to represent the cysteine and cysteamine conjugates of methacrylyl-CoA. The latter, a highly reactive species, was postulated to be the pathologic intermediate leading to physical malformations.

7.2 Nomenclature

No.	Disorder – affected component	Tissue distribution	Chromosomal location	MIM
7.1	Biotinidase deficiency	Serum (for diagnosis)	3p25	253260
7.2	Holocarboxylase synthetase deficiency	Leukocytes, cultured fibroblasts (for diagnosis)	21q22.1	253270
7.3	Propionic acidemia	Leukocytes, cultured fibroblasts (for diagnosis), liver		232000
	PCCA type A		13q32	232050
	PCCB type B		3q21–22	606054
7.4	3-Oxothiolase deficiency	Cultured fibroblasts (for diagnosis)	11q22.3–q23.1	203750
7.5	Methylmalonate semialdehyde dehydrogenase deficiency	Cultured fibroblasts (for diagnosis)	14q24.3	603178
7.6	3-Hydroxyisobutyric aciduria	Cultured fibroblasts (for diagnosis)	Unknown	236795
7.7	2-Methyl-3-hydroxybutyryl CoA dehydrogenase deficiency	Cultured fibroblasts, wbc (for diagnosis)	Unknown	_____
7.8	Methylmalonic acidemia	Leukocytes, cultured fibroblasts (for diagnosis), liver	6p21	251000
	Mut ⁰ , Mut ⁻			
	cblA		Unknown	251100
	cblB		Unknown	251100
7.9	Methylmalonic acidemia and homocystinuria cblC	Cultured fibroblasts (for diagnosis)	Unknown	277400
7.10	Methylmalonic acidemia and homocystinuria cblD	Cultured fibroblasts (for diagnosis)	Unknown	277410
7.11	3-Hydroxyisobutyryl-CoA deacylase deficiency	Cultured fibroblasts (for diagnosis)	Unknown	_____
7.12	2-Methylbutyryl CoA dehydrogenase deficiency	Cultured fibroblasts, wbc (for diagnosis)	10q25–q26	600301

7.3 Metabolic Pathways

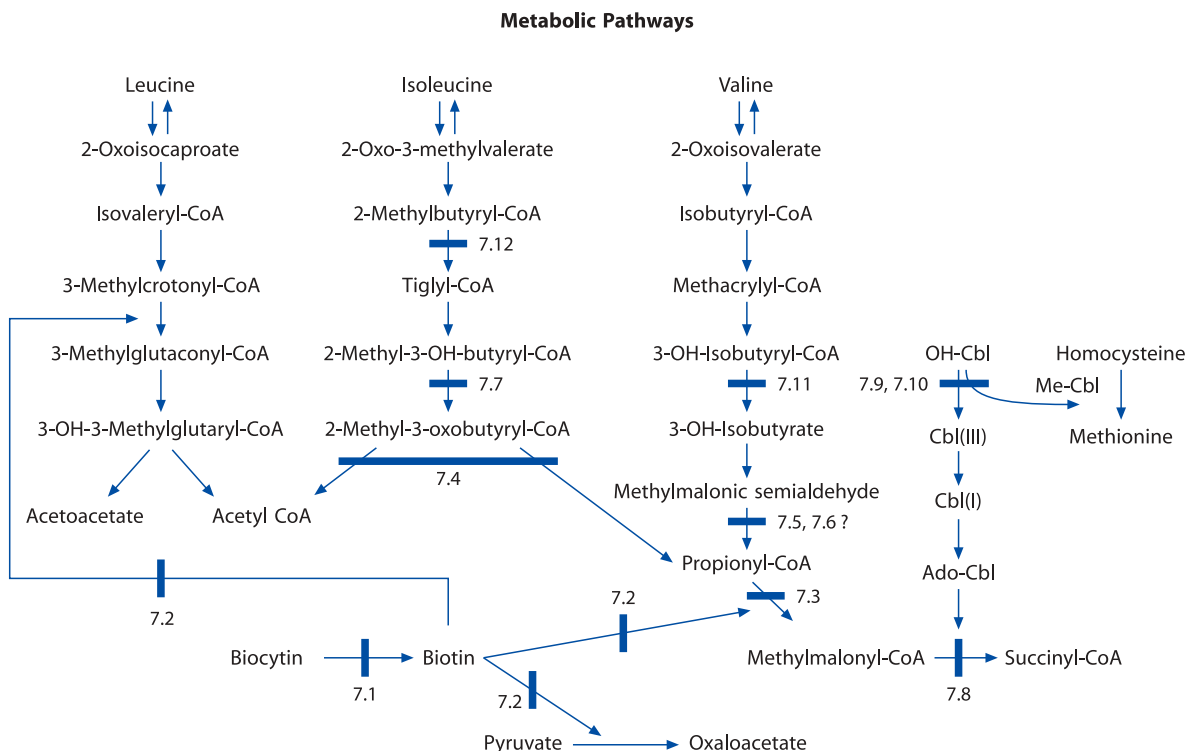


Fig. 7.1. Pathways of valine-isoleucine metabolism

7.1 The biotinidase reaction in which biocytin is cleaved to form biotin and lysine.

7.2 Holocarboxylase synthetase. This two-step reaction first activates biotin by forming the adenylate and then attaches the biotin moiety to the ϵ -amino group of a lysine on the carboxylase.

Multiple carboxylase deficiency is expressed in defective activity of pyruvate carboxylase, propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase in all tissues, including leukocytes and cultured fibroblasts.

7.3 The defect in propionic acidemia is in the enzyme propionyl-CoA carboxylase.

7.4 The 3-oxothiolase reaction and related products of the pathway.

7.5 The site of the defect in methylmalonic semialdehyde dehydrogenase deficiency.

7.6 3-Hydroxyisobutyric aciduria and no defined defect in the methylmalonate semialdehyde dehydrogenase.

- 7.7 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency.
 7.8 The methylmalonyl-CoA mutase reaction. The apoenzyme is defective in Mut methylmalonic acidemia. Methylmalonic aciduria is also seen with defective synthesis of adenosylcobalamin (Ado-Cbl) (Cbl A and B)
 7.9 Defective cobalamin metabolism leads to methylmalonic aciduria and homocystinuria (Cbl C).
 7.10 Defective cobalamin metabolism leading to methylmalonic aciduria and homocystinuria (Cbl D).
 7.11 3-Hydroxyisobutyryl-CoA deacylase deficiency.
 7.12 2-Methylbutyryl-CoA dehydrogenase deficiency.

7.4 Signs and Symptoms

Table 7.1. Biotinidase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Life-threatening illness early in life	+	+	+	+	+
	Ketoacidosis	+	+	+		
	Episodic vomiting	+	+	±	±	–
	Alopecia	+	+	+	+	+
	Periorificial cutaneous eruption	+	+	+	+	+
	Conjunctivitis	+	+	+	+	+
	Seizures	±	±	±	±	±
	Ataxia	–	+	+	+	+
	Spastic diplegia	–	–	+	+	+
	Hypotonia	+	+	–	–	–
	Optic atrophy	–	±	+	+	+
	Sensorineural deafness	–	±	+	+	+
	Monilial dermatitis	+	+	±	–	–
Routine laboratory	Acidosis	+	+	+	+	+
	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glucose (B)	↓ to n	↓ to n	↓ to n	↓ to n	↓ to n
	Ammonia	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
Special laboratory	Organic acids (U):					
	Lactate	↑	↑	↑	↑	↑
	3-Hydroxyisovalerate					
	3-Methylcrotonylglycine					
	3-Hydroxypropionate, methylcitrate	n to ↑	n to ↑	n to ↑	n to ↑	↑
CNS	Carboxylase activity (WBC)	↓	↓	↓	↓	↓
	Biotinidase activity	↓	↓	↓	↓	↓
	Developmental delay	±	±	±	±	±

Table 7.2. Holocarboxylase synthetase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Life-threatening illness, early in life	+	+	+	+	+
	Ketoacidosis, episodic vomiting	+	+	+	+	+
	Alopecia totalis	+	+	+	+	+
	Red scaly total body eruption	+	+	+	+	+
	Failure to thrive	±	±	±	±	±
	Moniliasis	+	+	+	+	+
	Immunodeficiency	±	+	+	±	±
	Acidosis	+	+	+	+	+
Routine laboratory	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glucose (B)	↓ to n	↓ to n	↓ to n	↓ to n	↓ to n
	Ammonia (B)	n to ↑	n to ↑	n	n	n
Special laboratory	Organic acids (U):					
	Lactate	↑	↑	↑	↑	↑
	3-Hydroxyisovalerate	↑	↑	↑	↑	↑
	3-Hydroxypropionate, methylcitrate, 3-methylcrotonylglycine	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	Carboxylase activity (WBC)(FB)	↓	↓	↓	↓	↓
	Holocarboxylase synthetase activity (WBC)(FB)	↓	↓	↓	↓	↓
CNS	Developmental delay	±	±	±	±	±

Table 7.3. Propionic acidemia

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Life-threatening illness	+	+	+	+	+
	Recurrent episodes of Ketosis	+	+	+	+	+
	Vomiting	+	+	+	+	+
	Failure to thrive	+	+	+	+	±
	Monilial dermatitis	+	+	±	±	±
	Hypotonicity	+	+	+	±	±
Routine laboratory	Neutropenia with or without thrombocytopenia or anemia	+	+	+	±	+
	Acidosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glucose (B)	n to ↓	n to ↓	n to ↓	n to ↓	n to ↓
	Ammonia (B)	n to ↑	n to ↑	n to ↑	n	n

Table 7.3 (continued)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Special laboratory-X-ray	Osteoporosis	–	+	+	+	+
	Organic acids (U): 3-Hydroxypropionate, methylcitrate, propionylglycine	↑	↑	↑	↑	↑
	Lactate	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	Propionyl-CoA carboxylase activity (WBC)(FB)	↓	↓	↓	↓	↓
	Glycine (B), propionyl carnitine (P)(U)	↑	↑	↑	↑	↑
CNS	Developmental delay	±	±	±	±	±
	Seizures	±	±	±	±	±
GI	Hepatomegaly	±	±	±	±	±

Table 7.4. 3-Oxothiolase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Acute episodes of ketosis and acidosis, vomiting and lethargy	+	+	+	+	+
Routine laboratory	Acidosis	+	+	+		+
	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glycine (B)	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	Ammonia (B)	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	Neutropenia, thrombocytopenia	±	±	±	±	±
Special laboratory	Organic acids (U) 2-Methyl-3-hydroxybutyrate, tiglylglycine, 2-methylacetate	↑	↑	↑	↑	↑
	3-Oxothiolase activity (FB)	↓	↓	↓	↓	↓
CNS	Developmental delay	±	±	±	±	±
	Seizures	±	±	±	±	±
Other	Failure to thrive	±	±	±	±	±

Table 7.5. 3-Hydroxyisobutyric acidemia due to methylmalonate semialdehyde dehydrogenase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Overwhelming illness	±	±	±		
	Failure to thrive, shortness of stature	±	±	±		
Routine laboratory	Vomiting	±	±	±		
	Anorexia	±	±	±		
	Acidosis	–	–	–		
	Ketosis	–	–	–		
	Anion gap	–	–	–		
	Ketoacidosis	±	±	±		
Special laboratory	Glucose (B)	n	n	n		
	Organic acids (U), 3-hydroxyisobutyric acid, 3-hydroxypropionic acid, 2-ethylhydracrylic acid	↑	↑	↑		
	Amino acids, (P)(U), methionine, 3-aminoisobutyric acid, β -alanine	↑	↑	↑		
	DNA mutation	+	+	+		
CNS	Developmental delay	±	±	±	±	±
GI	Hepatomegaly	±	±	±	±	±

Table 7.6. 3-Hydroxyisobutyric acidemia with normal methylmalonate semialdehyde dehydrogenase

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Episodic ketoacidosis, vomiting and dehydration	+	+	+	+	+
	Shortness of stature, failure to thrive	+	+	+	+	+
Routine laboratory	Acidosis	+	+	+	+	+
	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Lactate (B)	↑	↑	↑	↑	↑
Special laboratory	Organic acids (U): Lactate, 3-hydroxyisobutyrate, 2-ethyl-3-hydroxypropionate	↑	↑	↑	↑	↑
	Oxidation of ^{14}C -valine, β -alanine (FB)	↓	↓	↓	↓	↓

Table 7.7. 2-Methyl-3-hydroxybutyryl CoA dehydrogenase deficiency

System	Symptoms/marker	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Neurodegenerative disease	+	+			
Routine laboratory	Tachypnea	+	+			
	Acidosis	+	+			
	Ketosis	+	+			
	Glucose (B)	↓	↓			
Special laboratory	Organic acids (U):					
	Tiglylglycine	↑	↑			
	2-Methyl-3-hydroxybutyric acid	↑	↑			
	Tandem MS analysis (P):					
	C5-1-acylcarnitine	n-↑	n-↑	n-↑		
	C5-hydroxyacylcarnitine	n-↑	n-↑	n-↑		
	Hydroxyvanillic acid (C)	↑	↑	↑		
CNS	5-Hydroxyindole acetic acid (C)	↑	↑	↑		
	Mental retardation	±	+			
	Seizures	+	+			
Other	Choreoathetosis		+			
	Abnormal EEG	+	+			

Table 7.8. Methylmalonic acidemia

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Overwhelming illness	+	+	+	+	+
	Recurrent episodes of ketosis and acidosis	+	+	+	+	+
	Failure to thrive	+	+	+	+	±
	Vomiting	+	+	+	+	±
	Anorexia	+	+	+	±	±
	Monilial dermatitis	+	+	+	±	±
	Hypotonicity	+	+	+	±	±
Routine laboratory	Neutropenia with or without thrombocytopenia	+	+	+	+	+
	Acidosis	+	+	+	+	+
	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glucose (B)	↓ to n	↓ to n	↓ to n	↓ to n	↓ to n
Special laboratory	Ammonia (B)	n to ↑	n to ↑	n	n	n
	Osteoporosis	±	+	+	+	+
	Organic acids (U):					
	Methylmalonate	↑	↑	↑	↑	↑
	3-Hydroxypropionate, methylcitrate	↑	↑	↑	↑	↑
	Lactate	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	¹⁴ C-Propionate incorporation (FB)	↓	↓	↓	↓	↓
	Methylmalonyl-CoA mutase activity (FB)	↓	↓	↓	↓	↓

Table 7.8 (continued)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Complementation analysis	+	+	+	+	+
	Glycine (B)	↑	↑	↑	↑	↑
	Propionyl carnitine (P)(U)	↑	↑	↑	↑	↑
	Developmental delay	±	±	±	±	±
	Seizures	±	±	±	±	±
GI	Hepatomegaly	±	+	+	±	±

Table 7.9. Methylmalonic acidemia and homocystinuria cblC

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Overwhelming illness early in life	+	+	+		
	Hemolytic uremic syndrome	±	±	±		
	Failure to thrive	+	+	+	±	±
Routine laboratory	Anemia, megaloblastic	+	+	+	+	+
Special laboratory	Neutropenia	+	+	+	+	+
	Organic acids (U):					
	Methylmalonate	↑	↑	↑	↑	↑
	3-Hydroxypropionate, methylcitrate	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	Methionine (P)	↓	↓	↓	↓	↓
CNS	Total homocysteine (U)(P)	↑	↑	↑	↑	↑
	Mental retardation	+	+	+	+	+
	Ataxia	±	±	±	±	±
	Psychosis	±	±	±	±	±
	Seizures	±	±	±	±	±

Table 7.10. Methylmalonic acidemia and homocystinuria cblD

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Unique clinical findings	Overwhelming illness early in life	±	±	±	±	±
	Hemolytic uremic syndrome	±	±	±	±	±
	Failure to thrive	±	±	±	±	±
Routine laboratory	Anemia, megaloblastic	±	±	±	±	±
Special laboratory	Neutropenia	±	±	±	±	±
	Organic acids (U):					
	Methylmalonate	↑	↑	↑	↑	↑
	3-Hydroxypropionate, methylcitrate	n to ↑	n to ↑	n to ↑	n to ↑	n to ↑
	Methionine (P)	↓	↓	↓	↓	↓
CNS	Total homocysteine (U)(P)	↑	↑	↑	↑	↑
	Mental retardation	±	±	±	±	±
	Psychosis	±	±	±	±	±
	Ataxia	±	±	±	±	±
	Myelopathy	±	±	±	±	±

Table 7.11. 3-Hydroxyisobutyryl-CoA deacylase deficiency

System	Symptoms/markers	Neonatal	Infancy
Unique clinical findings	Multiple physical malformations	+	+
	Psychomotor retardation	+	+
	Dysmorphic facial features	+	+
	Vertebral abnormalities	+	+
	Cardiac malformations	+	+
	Cingulate gyrus-agenesis	+	+
	Corpus callosum-agenesis	+	+
Special laboratory	Sulfhydryl conjugates (U):		
	S-(2-carboxypropyl)cysteine	↑	↑
	S-(2-carboxypropyl)cysteamine	↑	↑
	3-Hydroxyisobutyryl-CoA deacylase activity	↓	↓
CNS	[¹⁴ C]-valine oxidation	↓	↓
	Feeding difficulties	+	+
	Hypotonia	+	+

Table 7.12. 2-Methylbutyryl-CoA dehydrogenase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood
Unique clinical findings	Muscular atrophy	±	±	±
	Mental retardation	±	±	±
	Lethargy	±	±	±
	Apnea	±	±	±
	Tachycardia	±	±	±
Routine laboratory	Glucose (B)	↓ to n	↓ to n	↓ to n
Special laboratory	Organic acids (U):			
	2-Methylbutyrylglycine	↑	↑	↑
	Tandem MS analysis (P):			
	C5-acylcarnitine	↑	↑	↑
CNS	2-Methylbutyryl-CoA dehydrogenase activity	↓	↓	↓
	Strabismus	±	±	±
	Abnormal MRI	±	±	±
	Abnormal EEG	±	±	±
	Hypothermia	±	±	±

7.5 Reference Values

■ Urinary Organic Acids (mmol/mol creatinine) (Gas Chromatography/Mass Spectrometry, GCMS)

Age	Lactate	3-Hydroxy- isovalerate	3-Methylcrotonyl- glycine	3-Hydroxy- propionate	Methylcitrate	Tiglylglycine	Propionylglycine	2-Methylbutyrylglycine	Methylmalonate	2-Methyl-3-hydroxybutyrate	3-Hydroxyisobutyrate	2-Ethyl-3-hydroxypropionate	2-Methylacetoacetate
All	<197	<2	<2	<24	<5	<2	<2	<2	<2	0–11	<24	<5	<2

■ Plasma Quantitative Amino Acids (μmol/l) (Ion-Exchange Column Chromatography) and Blood Lactate

Age	Glycine	Total homocysteine	Methionine	3-Aminoisobutyrate	Beta-alanine	Blood lactate (mmol/l)
Newborn	220–500	5–20	1–400	0	0–10	0.7–2
Child	100–400	5–20	2–59	0	0–7	0.7–2
Adult	120–550	5–20	6–40	0	0–12	0.7–2

■ Urinary Quantitative Amino Acids (mmol/mol creatinine)

Age	Glycine	Homocysteine
Newborn	100–400	0–0.01
Child – adult	3–340	0–0.01

■ Biotinidase Assay, Serum (nmol/min/ml)

Age	Biotinidase
All	4.3–7.5

■ Blood Acylcarnitine Profile (μmol/l)

Age	C3-acylcarnitine	C5-acylcarnitine	C5-hydroxyacylcarnitine	C5-1-acylcarnitine
All	<0.9	<0.4	<0.10	<0.10

■ CSF Neurotransmitters (HPLC) (μmol/l)

Age	Hydroxyvanillic acid	5-Hydroxyindole acetic acid
All	429–789	156–275

7.6 Pathological Values

Disorder	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	7.10	7.12
Lactate (U), times normal	<5	20–700				3–200					
3-Hydroxyisovalerate (U), times normal	<150	30–700									
3-Methylcrotonylglycine (U), times normal	<50	<120									
3-Hydroxypropionate (U), times normal	<3	19–500	10–200		2			4–650	<2	<2	
Methylcitrate (U), times normal	<10	4–80	100–500					6–100	50	50	
Tiglylglycine (U), times normal			100–1000	5–100			↑				
Propionylglycine (U), mmol/mol creat			100–1000					↑			
C3-Acylcarnitine (B), μmol/l			↑								
2-Methyl-3-hydroxybutyrate (U) (± 2-Methylacetoacetate), times normal				10–450			10–30				
3-Hydroxyisobutyrate (U), mmol/mol creat					3	180–390					
2-Ethylhydracrylate (2-ethyl-3-hydroxypropionate) (U), mmol/mol creat					19–85	19–85					
2-Methylbutyryl glycine (U), mmol/mol creat											3–21
C5-acylcarnitine (B) μmol/l											1.4–2.4
C5-1-acylcarnitine (B)							↑				
C5OH-acylcarnitine (B)							↑				
HVA/5-HIAA (CSF), μmol/l							824/330				
Methionine, β-alanine, β-aminoisobutyric acid (B) (U), times normal					5–50						
Methylmalonic acid (U), mmol/mol creat								20–1000	50–700	50–700	
Homocysteine (U), mmol/mol creat									0.08–80	0.08–80	

Disorder	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	7.10	7.12
Glycine (U), times normal			10–30					<7			
Methylmalonic acid (p), $\mu\text{mol/l}$								200–2500			
Glycine (P), times normal			5–10					<4			
Lactate (B), mmol/l						2.1–6.8					

Abnormalities for 7.11 are not shown, as detection of any amount of the sulfur conjugates associated with this disorder would be considered abnormal. Abbreviations: HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

7.7 Loading Tests

Loading tests are not applicable for 7.1, 7.2, 7.3, 7.6 and 7.7. In fact, in 7.3 and 7.6 a loading test would be disastrous. Loading tests are useful in 7.4 and 7.5.

An isoleucine challenge was performed in the patient with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, but it would appear unnecessary as the correct diagnosis can be obtained through other testing.

■ Disorder 7.4, Loading Test

Isoleucine 100 mg/kg p.o. Assay urine collected for 6 h for tiglylglycine and 2-methyl-3-hydroxybutyrate: 10–1000 and 300–4000 mmol/mol creatinine, respectively.

■ Disorder 7.5, Loading Test

Valine 100 mg/kg orally. Blood for glucose, lactate, electrolytes, 3-hydroxyisobutyrate, bicarbonate and valine at 0, 1, 2 and 4 h. Urine collected for 0–24 h in 8-h aliquots. Assay for organic acids and amino acids.

Normal response: no change in anything but valine concentration. Patient: ketoacidosis: HCO_3^- , 10 mEq/l; 3-hydroxybutyrate, 2–6 mmol/l. Modest decrease in glucose and increase in lactate in blood. Massive increase in 3-hydroxyisobutyrate in urine; over 30% of administered valine recovered as 3-hydroxyisobutyrate.

7.8 Diagnostic Flow Chart

In the diagnostic flow testing begins with the quantitative analysis of urinary organic acids by gas chromatography/mass spectrometry (GCMS). The initial specimen obtained is determined from the clinical symptomatology. In some patients, however, one must go directly to a more definitive assay. For instance, patients with biotinidase deficiency have been reported in whom organic acid analysis is normal. The decision to assay biotinidase must be based on clinical findings. Similarly, we have reported a patient with 3-oxothiolase deficiency in whom organic acid analysis, both sick and well, were normal. Only isoleucine loading elucidated the diagnosis. In addition, 3-hydroxyisobutyric aciduria may require valine loading. An alternative route would begin with tandem mass spectrometry in which 3-hydroxyisovaleryl carnitine would indicate multiple carboxylase deficiency; propionyl carnitine, propionic acidemia and methylmalonic acidemia; 2-methyl-3-hydroxybutyryl carnitine, oxothiolase deficiency. In most laboratories the next step would still be confirmation by GCMS, but in some instances, especially biotinidase deficiency and oxothiolase deficiency, it might be appropriate to go directly to enzymatic analysis. Patients with 2-methylbutyryl-CoA dehydrogenase and 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiencies illustrate the importance of accurate organic acid analysis, urine acylglycine conjugate analysis, and plasma acylcarnitine profiling.

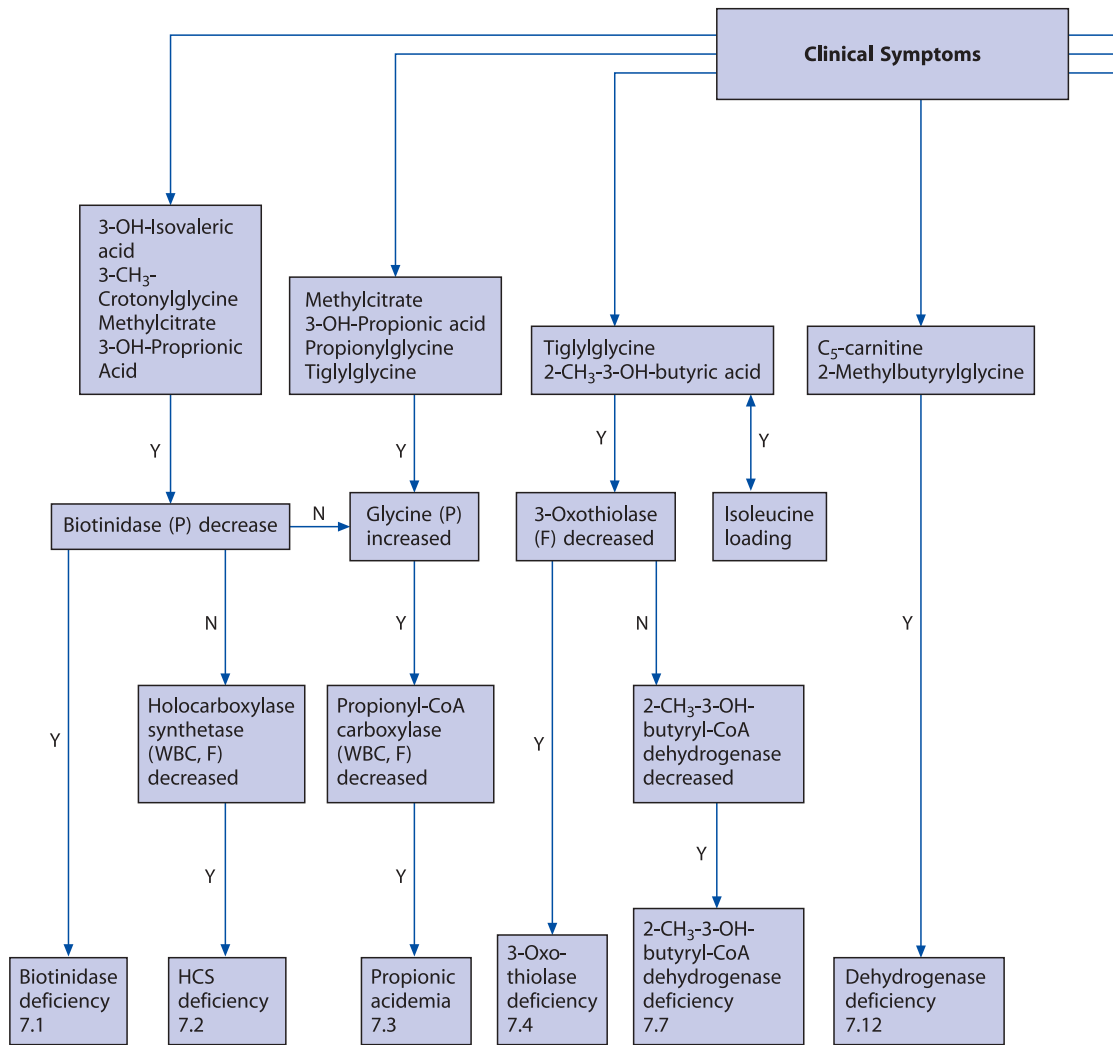
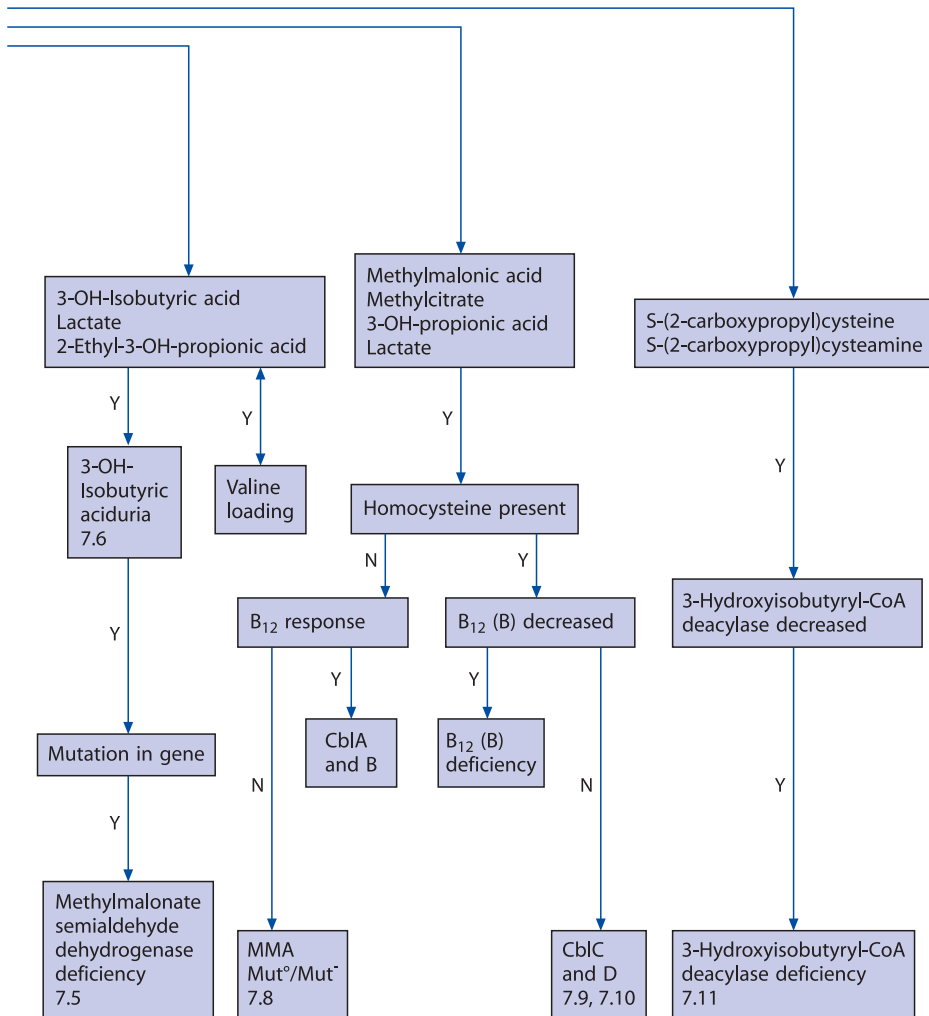


Fig. 7.2. Diagnostic flow chart



7.9 Specimen Collection

Tests	Sample requirements
Urine	5–20 ml (minimum 5 ml), frozen without preservatives and shipped frozen (packed in dry-ice) or lyophilized and shipped at room temperature with original volume specified
Amino acids	
Organic acids	
Carnitine	
Acylglycines	
Plasma	Plasma (0.5–1.0 ml) for each determination from heparinized blood (green-top tube), supernatant from clinical centrifugation (within 20 min), promptly frozen and shipped frozen (packed with dry-ice) or lyophilized and shipped at room temperature with original volume specified
Amino acids	
Organic acids	
Carnitine	
Biotinidase	
Acylcarnitine profile	
CSF	Cerebrospinal fluid 1 ml for each determination (standard plastic lumbar puncture tube or transferred to red-top tube), frozen and shipped frozen (packed with dry-ice)

7.10 Prenatal Diagnosis

Disorder	Material	Timing, trimester
7.1	Cultured amniocytes	II
7.2	Amniotic fluid	II
	Cultured amniocytes	II
7.3	Cultured amniocytes	II
	Amniotic fluid	II
7.4	Cultured amniocytes	II
	Amniotic fluid	II
7.8	Amniotic fluid	II
	Cultured amniocytes	II
7.9	Chorionic villus cells	I
	Cultured amniocytes	II
7.10	Chorionic villus cells	I
	Cultured amniocytes	II
7.12	Cultured amniocytes	II
	Amniotic fluid	II

7.11 DNA Analysis

Disorder	Material	Methodology
7.1	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.2	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.3	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.4	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.5	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.7	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.8	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP
7.12	F, WBC	RT-PCR; genomic amplification and sequencing; SSCP

RT-PCR, reverse transcription-polymerase chain reaction; SSCP, single-stranded conformational polymorphism analysis.

7.12 Initial Treatment

In each of these disorders the acute episode of acidosis is treated with a plentiful supply of water and electrolytes. An initial hydrating solution of isotonic NaHCO_3 with added potassium acetate and glucose is useful. Intravenous carnitine is a useful adjunct. In 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.12 a therapeutic diet is low in protein. Specific treatments follow:

- 7.1 and 7.2: biotin orally, 10 mg/day.
- 7.8: each patient should be tested for B_{12} responsiveness by a trial of parenteral hydroxycobalamin in a dose of 1 mg/day. In those that respond, therapy is continued but the oral route may be used. Cyanocobalamin may be used if hydroxycobalamin is not available.

7.13 Summary/Comments

The disorders of isoleucine and valine metabolism are detected in a sequential process that begins with the evaluation of the symptoms and signs displayed by the patient. Clinical chemistry is helpful in the assessment of ketogenesis by urinary tests for ketones or quantification of 3-hydroxybutyrate and acetoacetate in the blood. The electrolytes and pH may provide evidence of acidosis and it is important to assess the presence or absence of hyperammonemia. Amino acid analysis of the plasma and urine may be helpful. In virtually all instances the definitive diagnosis will come from organic acid analysis of the urine.

A simple colourimetric test for biotinidase deficiency permits incorporation into programs of routine neonatal screening. Treatment with biotin is effective in preventing or curing most of the manifestations of the disease.

Exceptions appear to be the optic and auditory nerve atrophy and spastic diplegia.

Quantification of the organic acids of the urine is important in diagnosis. At the acute crisis many compounds accumulate which may suggest alternate diagnoses. For example, 2-methylacetoacetate and 2-methyl-3-hydroxybutyrate found in a patient with propionic acidemia suggest a diagnosis of 2-oxothiolase deficiency. Occasional elevation of 3-hydroxyisovalerate in such a patient suggests a diagnosis of multiple carboxylase deficiency, and a decrease with therapy may mistakenly suggest a response to biotin. A patient sent home with the kind of protein-containing diet employed in biotin-responsive multiple carboxylase deficiency might not survive a second acute episode of the ketoacidosis associated with propionic acidemia. Quantification resolves these issues, because these metabolites are minor ones in propionic acidemia compared to methylcitrate and 3-hydroxypropionate. In contrast, in multiple carboxylase deficiency 3-hydroxyisovalerate is a major component and methylcitrate a minor one.

The GCMS analysis of methylcitrate or methylmalonate in the amniotic fluid is of advantage over enzyme analysis because of its rapidity. Moreover, it avoids possible maternal contamination of amniocyte cultures. In one documented instance the fetus was diagnosed as affected by methylcitrate analysis and normal by enzyme analysis; an affected infant was born and the cells ultimately available for analysis were later shown to be maternal. In families in which the mutation is known, prenatal diagnosis may be carried out by analysis of DNA, but the numbers of mutations are such that this is not generally available.

Among the organic acidemias, 3-oxothiolase deficiency is the one that is likely to remain undiagnosed even after organic acid analysis of the urine has been performed. At the time of acute ketoacidosis there may be little or no tiglylglycine or 2-methyl-3-hydroxybutyrate in the urine, and the latter may be elevated amounts in the urine of anyone who is ketotic. With resolution of the acute illness, it is not unusual for urine organic acid analysis to be normal. The isoleucine load is invaluable in this situation.

2-Methylbutyryl-CoA dehydrogenase deficiency appears to be another inborn error of metabolism which does not become disease per se unless the patient undergoes some level of metabolic stress. Of two affected siblings, the first manifested neurologic sequelae following a probable ischemic/hypoxic event at 3 days of age. His sister, identified prenatally, has been completely asymptomatic.

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