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6.1 Introduction

The inborn errors of L-leucine catabolism present biochemically with branched-chain amino and/or organic aciduria [1]. These disorders include maple syrup disease (MSD; branched-chain α -ketoacid dehydrogenase (BCKD) deficiency), isovaleric acidemia (isovaleryl-coenzyme A (CoA) dehydrogenase deficiency), isolated 3-methylcrotonyl-CoA carboxylase deficiency, the 3-methylglutaconic acidurias (3-methylglutaconyl-CoA hydratase deficiency, Barth syndrome, and other disorders in which the primary defect has not been demonstrated), and 3-hydroxy-3-methylglutaric aciduria (3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase deficiency).

The prevalence of MSD is approximately 1 in 200 000 persons and is most common among the Mennonites of North America where the incidence is 1 in 380. Although all three branched-chain amino acids, leucine, isoleucine, and valine and their respective α -ketoacids are increased in blood, urine and cerebrospinal fluid [2], it is the elevated leucine levels that are responsible for the clinical pathogenesis of the disorder. There are four forms, which differ in the age and severity of onset, biochemical findings, and responsiveness to thiamin (vitamin B₁), a cofactor for the BCKD complex. The classical form presents in the first week of life with poor feeding, irritability and lethargy with progressive central nervous system deterioration; the intermediate form presents at any age, infancy to adulthood, with failure to thrive, neurologic features and ketoacidosis; an intermittent form manifests episodic ataxia and ketoacidosis, often associated with increased protein consumption or intercurrent illness; and a 'thiamin-responsive form' exists in which metabolic abnormalities are ameliorated with large doses of thiamin. Urine spot testing with 2,4-dinitrophenylhydrazine (DNPH) and ferric chloride will indicate the presence of oxoacids, and the diagnosis is confirmed with plasma or serum quantitative amino acid and urine organic acid analysis. Newborn screening for the classical form of MSD, employing measurement of leucine levels in dried blood filter paper spots, is available in many locations. Although many patients have psychomotor handicaps, there are increasing reports of patients with normal development when treatment was started in the first few days of life. At-risk

neonates can be diagnosed between ages 12–24 hours by amino acid quantification using high-performance liquid chromatography (HPLC) or tandem mass spectrometry (MS/MS). After delivery in affected infants there is an increase in plasma leucine caused by postpartum endogenous protein catabolism and a characteristic decrease in plasma alanine. Affected neonates ($n=19$) have serum or plasma leucine concentrations 233–733 $\mu\text{mol/l}$ (nl 43–186), alanine concentrations range from 35–285 $\mu\text{mol/l}$ (nl 206–545), with molar ratios of [Leu]/[Ala] of 1.3–12.4 (nl 0.1–0.4). In older, more severely intoxicated neonates, the leucine to alanine molar ratio is markedly abnormal, range 5–97.

The remaining disorders of L-leucine catabolism are less common than MSD and characterized almost exclusively by branched-chain organic aciduria [1–3]. Patients with isovaleric acidemia (<100 cases) present either with a severe, neonatal form during the first two weeks of life, or with a chronic intermittent form during the first year of life [4–6]. Approximately one-half of patients with the severe neonatal form do not survive. A distinctive odor characterized as ‘sweaty feet’ can be noted in body secretions during acute episodes. Although many patients have psychomotor handicaps, normal development has been reported. Myelodysplasia of bone marrow and arrest of the myeloid series at the promyelocytic stage has suggested acute promyelocytic leukemia (APL) in some patients [5]; moreover, for those patients presenting in coma, excess sensitivity (decreased blood glucose) upon insulin intervention has suggested diabetic coma. The differential diagnosis includes multiple acyl-CoA dehydrogenase deficiency (so called glutaric aciduria type II), but careful organic acid profiling in urine can provide the correct differential diagnosis.

Patients with isolated 3-methylcrotonyl-CoA carboxylase deficiency (<40 patients) present with considerable phenotypic heterogeneity while biochemical findings are more consistent [7–10]. Many patients present after the first year of life; some are asymptomatic at the time of diagnosis and come to attention only following identification of symptoms in a sibling or identification of abnormal acylcarnitine profile in blood relatives using tandem mass spectrometry. In most symptomatic patients, the clinical presentation consists of sudden (acute) episodes of Reye-like disease, with vomiting, hypotonia, seizures, and coma. An acrid odor of the urine has been noted. Progressive respiratory insufficiency leading to respiratory failure has been increasingly observed.

The range of clinical and biochemical findings in the inherited 3-methylglutaconic acidurias is extensive [11, 12]. All patients excrete elevated 3-methylglutaconic and 3-methylglutaric acids. Patients with 3-methylglutacetyl-CoA hydratase deficiency (so called ‘type I’; <15 patients) excrete increased 3-hydroxyisovaleric acid, which is useful in the differential diagnosis. Phenotypic expression has ranged from benign to a severe neurologic disease. Other forms of 3-methylglutaconic aciduria are well-defined clini-

cally, but the primary metabolic defect is unknown [13–15]. Barth syndrome (so called ‘type II’ 3-methylglutaconic aciduria) is an X-linked disorder characterized by skeletal myopathy, dilated cardiomyopathy, proportionate short stature, recurrent neutropenia and mild hypocholesterolemia. Neuromuscular and cardiovascular symptoms and the severity of recurring infections tend to improve with age [16, 17]. It has been suggested that 3-methylglutaconic aciduria in Barth syndrome is an epiphenomenon which does not reflect the primary defect. Costeff optic atrophy syndrome (so called ‘type III’ 3-methylglutaconic aciduria) is a movement disorder with similarities to the syndrome described by Behr [18, 19]. It has thus far been identified only in Iraqi Jews who emanate from a distinct region near Baghdad, Iraq. The remaining patients with 3-methylglutaconic aciduria (so called ‘type IV’ or unclassified form) manifest a wide range of neurologic, peripheral organ, and metabolic disturbances, and will likely be subcategorized into distinct phenotypes as additional patients and candidate genes are identified [11, 12].

Several patients with idiopathic (type IV) 3-methylglutaconic aciduria have abnormalities in respiratory chain function, or were diagnosed with Leigh syndrome. Of interest, one patient with isolated 3-methylglutaconic aciduria/3-hydroxy-3-methylglutaric aciduria manifested combined deficiency of complexes II/III of the respiratory chain. Therapeutically, intervention with pantothenic acid in several patients has resulted in significant improvement of cardiac function. Several patients with idiopathic (type IV?) 3-methylglutaconic aciduria manifested clinical improvement during coenzyme Q₁₀ intervention, whereas coenzyme Q₁₀ therapy was without clinical benefit in patients with Costeff optic atrophy syndrome.

The constellation of metabolic acidosis, hypoketotic hypoglycemia, vomiting, lethargy and a characteristic urinary organic acid profile is typified in patients with HMG-CoA lyase deficiency [20–22]. Of interest, this is the only disorder in the distal pathway of L-leucine catabolism for which an animal model (knockout mouse) has been developed [23]. The mouse model, unexpectedly, is an embryonic lethal, showing abnormal mitochondria and marked hepatic vacuolization. HMG-CoA lyase has also been shown to be targeted both to mitochondria and peroxisomes, perhaps indicating a role for both organelles in hepatic ketogenesis [24]. HMG-CoA lyase deficiency has also been recently associated with Down syndrome and VATER syndrome (vertebral defects, anal atresia, tracheoesophageal fistula, radial upper limb hypoplasia, and renal defects). Emerging experience with MRI suggests that a combination of diffuse mild and multifocal more serious cerebral white matter abnormalities is common in HMG-CoA lyase deficiency; these abnormalities no doubt relate to length of exposure and severity of hypoglycemic episodes.

The disorders of the leucine pathway, including MSD, can usually be diagnosed by urine organic analysis. MSD is the only disorder of the pathway

that causes elevated blood leucine. MSD is usually diagnosed by plasma or serum amino acid quantitation but can be recognized clinically by the features of ketonuria, encephalopathy, and the distinctive odor of maple syrup or burnt sugar or cerumen. For most of the disorders of the pathway, the management of the disorder requires initial correction of acidosis, suppression of endogenous protein catabolism by glucose infusion, enteral or intravenous L-carnitine therapy (except 6.1) and restriction of dietary leucine/protein. In 6.4, all ketogenic amino acids and fatty acids contribute to the generation of HMG acid, and acute management requires suppression of protein and fatty acid catabolism and subsequent dietary restriction of protein and fat.

6.2 Nomenclature

No.	Disorder-affected component	Tissue distribution	Chromosomal location	McKusick
6.1	Maple syrup disease (branched-chain α -ketoacid dehydrogenase complex deficiency)	WBC, FB		248600 248610 248611
	1. Decarboxylase (E_1)			
	(a) E_1 α -subunit		19q13.1–13.2	
	(b) E_1 β -subunit		6p21–22	
	2. Dihydrolipoyl acyl-transferase (E_2)		1p21–31	
	3. Lipoamide dehydrogenase (E_3)		7q31	
6.2	Isovaleric acidemia (isovaleryl-CoA dehydrogenase deficiency)	WBC, FB	15q14–q15	243500
6.3	3-Methylcrotonyl-CoA carboxylase deficiency	WBC, FB	MCCA: 3p11.2–p13 MCCB: 5q12–q13.2	210200
6.4	3-Methylglutaconic aciduria type I (3-methylglutaconyl-CoA hydratase deficiency)	WBC, FB		250950
6.5	Barth syndrome, type II 3-methylglutaconic aciduria	WBC, FB, muscle	Xq28	302060
6.6	Costeff optic atrophy syndrome ^a , type III 3-methylglutaconic aciduria		19q13.2–q13.3	258501
6.7	3-Methylglutaconic aciduria, idiopathic (type IV)			250951
6.8	3-OH-3-methylglutaric aciduria (3-OH-3-methylglutaryl-CoA lyase deficiency)	WBC, PLT, FB	1p35–36	246450

^a Primary defect not conclusively identified in Costeff optic atrophy syndrome and idiopathic 3-methylglutaconic aciduria.

6.3 Metabolic Pathway

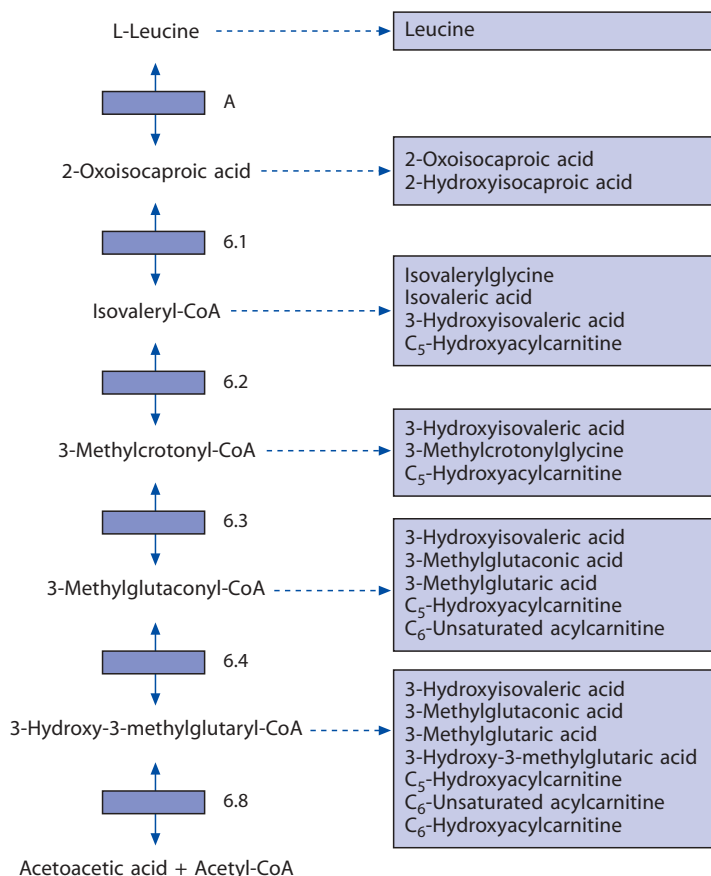


Fig. 6.1. The L-leucine degradative pathway. Reactions for which inherited metabolic disorders have not been conclusively identified include A, leucine-isoleucine aminotransferase and the majority of the 3-methylglutaconic acidurias (6.6–6.7). 6.1, Branched-chain α -ketoacid dehydrogenase (BCKD) complex, a reaction also occurring in the initial steps of L-isoleucine and L-valine degradation; 6.2, isovaleryl-CoA dehydrogenase; 6.3, 3-methylcrotonyl-CoA carboxylase; 6.4, 3-methylglutaconyl-CoA hydratase; 6.8, HMG-CoA lyase. Pathologic urinary metabolites used as specific markers in the differential diagnosis are presented in squares. Abbreviation: CoA, coenzyme A

6.4 Signs and Symptoms

Table 6.1. Maple syrup disease (all forms)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Episodic vomiting	+	+	+	+	+
	Lethargy	+	+	+	+	+
	Coma	±	±	±	±	±
	Odor of maple syrup	±	±	±	±	±
Routine laboratory	Acidosis	+	+	+	+	+
	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glucose (B)	↓-n	↓-n	↓-n	↓-n	↓-n
	Ammonia (B)	n-↑	n-↑	n-↑	n-↑	n-↑
Special laboratory	2,4-Dinitrophenylhydrazine test (U)	±	±	±	±	±
	Ferric chloride test (U)	±	±	±	±	±
	Branched-chain amino acids (P or S)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	Organic acids: branched-chain oxoacids (ketoacids) (P, S or U)	↑↑-↑↑↑	↑↑-↑↑↑	↑↑-↑↑↑	↑↑-↑↑↑	↑↑-↑↑↑
	Psychomotor retardation	±	±	±	±	±
CNS	CNS deterioration	±	±	±	±	±
	Cerebral edema	±	±	±	±	±
	Areflexia	±	±	±	±	±
	Hypotonia	±	±	±	±	±
	Hypertonia	±	±	±	±	±
	Ataxia	±	±	±	±	±
	Seizures	±	±	±	±	±
Other	Irritability	±	±	±	±	±
	Apnea	±	±	±	±	±
	Poor feeding	±	±	±	±	±
	Failure to thrive	±	±	±	±	±

Table 6.2. Isovaleric acidemia

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Episodic vomiting	±	±	±	±	±
	Lethargy	±	±	±	±	±
	Coma	±	±	±	±	±
	Odor of 'sweaty feet'	±	±	±	±	±
	Hypothermia	±	±	±	±	±
Routine laboratory	Acidosis	+	+	+	+	+
	Ketosis	+	+	+	+	+
	Anion gap	+	+	+	+	+
	Glucose (B)	↓-n	↓-n	↓-n	↓-n	↓-n
	Ammonia (B)	n-↑	n-↑	n-↑	n-↑	n-↑
	Uric acid (B)	n-↑	n-↑	n-↑	n-↑	n-↑
	Calcium (B)	↓-n	↓-n	↓-n	↓-n	↓-n
	Neutropenia	±	±	±	±	±
	Thrombocytopenia	±	±	±	±	±
	Pancytopenia	±	±	±	±	±
	Hypoplasia of hematopoietic cell lines	±	±	±	±	±
Special laboratory	Organic acids: isovalerylglutamine and its metabolites (U)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	3-Hydroxyisovaleric acid	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	Glycine (P)	n-↑	n-↑	n-↑	n-↑	n-↑
	C ₅ -acylcarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	Volatile short-chain organic acids: isovaleric acid (P)	n-↑	n-↑	n-↑	n-↑	n-↑
	Carnitine; total and free (P)	↓	↓	↓	↓	↓
	Carnitine; esterified (P)	↑	↑	↑	↑	↑
CNS	Psychomotor retardation	±	±	±	±	±
	Seizures	±	±	±	±	±
Other	Natural aversion to protein foods	±	±	±	±	±
	Cholestasis	±	±	±	±	±
	Alopecia	±	±	±	±	±

Table 6.3. Isolated 3-methylcrotonyl-CoA carboxylase deficiency

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Episodic vomiting	±	±	±	±	
	Lethargy	±	±	±	±	
	Subcoma/coma	±	±	±	±	
	Somnolence/sopor	±	±	±	±	
	Diarrhea	±	±	±	±	
	Urine – acrid odor	±	±	±	±	
	Respiratory infections/insufficiency	±	±	±	±	
	Hepatosplenomegaly	±	±	±	±	
Routine laboratory	Failure to thrive	±	±	±	±	
	Acidosis	±	±	±	±	
	Ketosis	±	±	±	±	
	Ammonia (B)	n-↑	n-↑	n-↑	n-↑	
	Glucose (B)	↓-n	↓-n	↓-n	↓-n	
	Base excess/anion gap	±	±	±	±	
	Neutrophilia	±	±	±	±	
	Thrombocytopenia	±	±	±	±	
	Aspartate transaminase (ASAT) (S)	n-↑	n-↑	n-↑	n-↑	
	Alanine transaminase (ALAT) (S)	n-↑	n-↑	n-↑	n-↑	
Special laboratory	Uric acid (B)	n-↑	n-↑	n-↑	n-↑	
	Organic acids: 3-hydroxyisovaleric acid and 3-methylcrotonylglycine (U)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	
	Carnitine; total and free (P)	↓-n	↓-n	↓-n	↓-n	
	Carnitine; esterified (P)	n-↑	n-↑	n-↑	n-↑	
CNS	C ₅ -hydroxyacylcarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	
	Psychomotor retardation	±	±	±	±	
	Cerebral edema	±	±	±	±	
	Seizures	±	±	±	±	
	Hyperreflexia	±	±	±	±	
	Hypertonia	±	±	±	±	
	Hypotonia	±	±	±	±	
	Spastic paraplegia/tetraplegia	±	±	±	±	
	Opisthotonus	±	±	±	±	
	Cerebral atrophy	±	±	±	±	
	Nystagmus	±	±	±	±	
	Involuntary movements	±	±	±	±	
	Hemiparesis	±	±	±	±	
	Hemilateral focal edema	±	±	±	±	
	Gliosis	±	±	±	±	
	Abnormal MRI	±	±	±	±	
	Abnormal EEG	±	±	±	±	
	Speech delay	±	±	±	±	
	Mild ataxia	±	±	±	±	
	Flexor spasms	±	±	±	±	
	Myopathy	±	±	±	±	

Table 6.3 (continued)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Other	Apnea	±	±	±	±	
	Tachypnea	±	±	±	±	
	Cardiomyopathy	±	±	±	±	
	Fatty deposition in liver	±	±	±	±	
	GER (gastroesophageal reflux)	±	±	±	±	
	Esophageal peristalsis	±	±	±	±	
	Diaphragmatic paresis	±	±	±	±	
	Hypsarrhythmia	±	±	±	±	

Table 6.4. 3-Methylglutaconic aciduria, type I (3-methylglutaconyl-CoA hydratase deficiency)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Delayed language development	±	±	±		
	Respiratory infections	±	±	±		
	Vomiting	±	±	±		
	Coma	±	±	±		
Routine laboratory	Acidosis	±	±	±		
	CK (S or P)	n-↑	n-↑	n-↑		
	Thrombocytopenia	±	±	±		
	Glucose (B)	↓-n	↓-n	↓-n		
	Ammonia (B)	n-↑	n-↑	n-↑		
	Aspartate transaminase (ASAT) (S)	n-↑	n-↑	n-↑		
	Alanine transaminase (ALAT) (S)	n-↑	n-↑	n-↑		
Special laboratory	Organic acids: 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids (U)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑		
	Carnitine; esterified fraction (P)	n-↑	n-↑	n-↑		
	Carnitine; total and free (P)	↓-n	↓-n	↓-n		
	C ₅ -hydroxyacylcarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑		
	C ₆ -unsaturated acylcarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑		
CNS	Abnormal MRI	±	±	±		
	Psychomotor retardation	±	±	±		
	Peripheral hypotonia	±	±	±		
	Axial hypertonia	±	±	±		
	Diffuse white matter disease	±	±	±		
	Dysmyelination	±	±	±		
	Hyperintense areas in basal ganglia	±	±	±		
	Extrapyramidal signs	±	±	±		
	Macrocephaly	±	±	±		
	Seizures	±	±	±		
	Abnormal CT scan	±	±	±		
	Altered consciousness	±	±	±		
	Decerebrate posture	±	±	±		
	Involuntary movements	±	±	±		
	Spastic quadriplegia	±	±	±		
	Self-mutilation	±	±	±		

Table 6.4 (continued)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Other	Insomnia	±	±	±		
	Irritability	±	±	±		
	Head lag	±	±	±		
	Gastroesophageal reflux	±	±	±		
	Hepatomegaly	±	±	±		
	Tachypnea	±	±	±		
	Bronchiolitis	±	±	±		
	Failure to thrive	±	±	±		
	Scoliosis	±	±	±		
	Emotional outbursts (crying/ screaming fits)	±	±	±		

Table 6.5. Barth syndrome (X-linked 3-methylglutaconic aciduria, normal 3-methylglutaconyl-CoA hydratase activity)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Dilated cardiomyopathy	+	+	+	+	+
	Growth retardation (short stature)	+	+	+	+	+
	Cardioskeletal myopathy	+	+	+	+	+
	Cyclic neutropenia	+	+	+	+	+
	Endocardial fibroelastosis	+	+	+	+	+
Routine laboratory	Cholesterol (P)	↓-n	↓-n	↓-n	↓-n	↓-n
	Uric acid (B)	n-↑	n-↑	n-↑	n-↑	n-↑
Special laboratory	Organic acids: 3-methylglutaconic and 3-methylglutaric acids (U)	↑	↑	↑	↑	↑
	2-Ethylhydracrylic acid (U)	n-↑	n-↑	n-↑	n-↑	n-↑
CNS	Hypertelorism	±	±	±	±	±
Other	Polydactyly	±	±	±	±	±
	Abnormal auricles	±	±	±	±	±

Table 6.6. Costeff optic atrophy syndrome (3-methylglutaconic aciduria, normal 3-methylglutaconyl-CoA hydratase activity)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Optic atrophy	+	+	+	+	+
	Movement disorder	+	+	+	+	+
	Spastic paraplegia	±	±	±	±	±
	Ataxia	±	±	±	±	±
	Cognitive deficiency	±	±	±	±	±
	Dysarthria	±	±	±	±	±
	Choreoathetosis	±	±	±	±	±
Special laboratory	Organic acids: 3-methylglutaconic and 3-methylglutaric acids (U)	↑	↑	↑	↑	↑
CNS	Hyperreflexia	±	±	±	±	±
	Hypotonia	±	±	±	±	±
	Ankle clonus	±	±	±	±	±

Table 6.7. 3-Methylglutaconic aciduria, idiopathic (normal 3-methylglutaconyl-CoA hydratase activity)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Dilated cardiomyopathy	±	±	±	±	±
	Hypertrophic cardiomyopathy	±	±	±	±	±
	Recurrent infections	±	±	±	±	±
	Endocardial fibroelastosis	±	±	±	±	±
	Dementia	±	±	±	±	±
	Deafness	±	±	±	±	±
	Blindness	±	±	±	±	±
	Failure to thrive	±	±	±	±	±
	Spastic quadriplegia	±	±	±	±	±
	Arrested development	±	±	±	±	±
Routine laboratory	Ketoacidosis	±	±	±	±	±
	Glucose (B)	↓-n	↓-n	↓-n	↓-n	↓-n
	Creatine kinase (S or P)	n-↑	n-↑	n-↑	n-↑	n-↑
	Ammonia (B)	n-↑	n-↑	n-↑	n-↑	n-↑
	Aspartate transaminase (ASAT) (S)	n-↑	n-↑	n-↑	n-↑	n-↑
	Alanine transaminase (ALAT) (S)	n-↑	n-↑	n-↑	n-↑	n-↑
Special laboratory	Macrocytic anemia	±	±	±	±	±
	Organic acids: 3-methylglutaconic and 3-methylglutaric acids (U)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	Organic acids: tricarboxylic acid cycle intermediates (U)	n-↑↑↑	n-↑↑↑	n-↑↑↑	n-↑↑↑	n-↑↑↑
	Carnitine: free and total (P)	↓-n	↓-n	↓-n	↓-n	↓-n
	Lactic acid (U)	n-↑	n-↑	n-↑	n-↑	n-↑
	Methionine (B)	n-↑↑	n-↑↑	n-↑↑	n-↑↑	n-↑↑
	Hepatic lipid	n-↑	n-↑	n-↑	n-↑	n-↑
	Cardiac/skeletal muscle:					
	Lipid	n-↑	n-↑	n-↑	n-↑	n-↑
	Glycogen	n-↑	n-↑	n-↑	n-↑	n-↑
CNS	Hyperreflexia	±	±	±	±	±
	Spastic paraparesis	±	±	±	±	±
	Extrapyramidal signs	±	±	±	±	±
	Psychomotor retardation	±	±	±	±	±
	Hypertonia	±	±	±	±	±
	Hypotonia	±	±	±	±	±
	Seizures	±	±	±	±	±
	Facial myopathy	±	±	±	±	±
	Cerebellar findings (ataxia, hypoplasia, dysgenesis)	±	±	±	±	±
	Nystagmus	±	±	±	±	±
Other	Leigh syndrome	±	±	±	±	±
	Progressive encephalopathy	±	±	±	±	±
	Rigidity	±	±	±	±	±
	Abnormal MRI	±	±	±	±	±
	Dysmorphic features	±	±	±	±	±
	Hepatic dysfunction	±	±	±	±	±
	Pancreatitis	±	±	±	±	±
	Nasal quality to speech	±	±	±	±	±

Table 6.7 (continued)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
	Hepato(spleno)megaly	±	±	±	±	±
	Febrile episodes	±	±	±	±	±
	Cervical lymphadenopathy	±	±	±	±	±
	Progressive decrease in physical performance	±	±	±	±	±

Table 6.8. 3-OH-3-methylglutaric aciduria (3-OH-3-methylglutaryl-CoA lyase deficiency)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Episodic vomiting	±	±	±	±	±
	Lethargy	±	±	±	±	±
	Coma	±	±	±	±	±
	Altered respiration (tachypnea, hyperpnea, dyspnea)	±	±	±	±	±
	Cardiomyopathy	±	±	±	±	±
	Abnormal MRI	±	±	±	±	±
	Brain edema	±	±	±	±	±
Routine laboratory	Acidosis	+	+	+	+	+
	Hypoketotic hypoglycemia	+++	+++	+++	+++	+++
	Aspartate transaminase (ASAT) (S)	n-↑	n-↑	n-↑	n-↑	n-↑
	Alanine transaminase (ALAT) (S)	n-↑	n-↑	n-↑	n-↑	n-↑
	Ammonia (B)	n-↑	n-↑	n-↑	n-↑	n-↑
	Cyanosis	±	±	±	±	±
	Hepatic lipid	n-↑	n-↑	n-↑	n-↑	n-↑
Special laboratory	Hypochromic microcytic anemia	±	±	±	±	±
	Organic acids: 3-hydroxyisovaleric, 3-methylglutaconic, 3-methylglutaric and 3-OH-3-methylglutaric acids (U)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	Organic acids: 3-methylcrotonylglycine, dicarboxylic acids (glutaric, adipic, sebacic and suberic acids) (U)	n-↑	n-↑	n-↑	n-↑	n-↑
	C ₅ -hydroxyacylcarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	C ₆ -unsaturated acylcarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	C ₆ -dicarboxylic monocarnitine (P)	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑	↑-↑↑↑
	Carnitine: free fraction (P)	↓-n	↓-n	↓-n	↓-n	↓-n
CNS	Carnitine: esterified fraction (P)	n-↑	n-↑	n-↑	n-↑	n-↑
	Mental retardation	±	±	±	±	±
	Cerebral atrophy	±	±	±	±	±
	Convulsions	±	±	±	±	±
	Hypertonia	±	±	±	±	±
	Hypotonia	±	±	±	±	±
	Hyperreflexia	±	±	±	±	±
	Macrocephaly	±	±	±	±	±
	White matter lesions	±	±	±	±	±

Table 6.8 (continued)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Other	Aphasia	±	±	±	±	±
	Facial palsy	±	±	±	±	±
	Bilateral occipital porencephaly	±	±	±	±	±
	Bilateral sensorineural deafness	±	±	±	±	±
	Retinitis pigmentosa	±	±	±	±	±
	Abnormal EEG	±	±	±	±	±
	Tapeto-retinal degeneration	±	±	±	±	±
	Spastic tetraplegia	±	±	±	±	±
	Hepatomegaly	±	±	±	±	±
	Diarrhea	±	±	±	±	±
	Irritability	±	±	±	±	±
	Pancreatitis	±	±	±	±	±
	Natural aversion to protein foods	±	±	±	±	±
	Gastroenteritis	±	±	±	±	±

6.5 Reference Values

■ Urine/Spot Screening Tests

	2,4-Dinitrophenylhydrazine (DNPH) test	Ferric chloride test	Newborn screening
Normals	No precipitate	No color change	Leucine <2 mg/dl (<153 $\mu\text{mol/l}$)

■ Plasma Quantitative Amino Acids ($\mu\text{mol/l}$) (Ion Exchange Column Chromatography or High-Performance Liquid Chromatography, HPLC)

Age	Valine	Isoleucine	Leucine	Alloisoleucine
Premature (first 6 weeks)	99–220	23–85	151–200	0
0–1 month	86–190	26–91	48–160	0
1–24 months	64–294	31–86	47–155	0
2–18 yrs	74–321	22–107	49–216	0
Adult	119–336	30–108	72–201	0

■ Urine Organic Acids (mmol/mol Creatinine) (Gas Chromatography/Mass Spectrometry, GC/MS)

Age	2-Oxoisocaproic acid	2-Oxo-3-methylvaleric acid	2-Oxoisovaleric acid	2-Hydroxyisovaleric acid	2-Hydroxyisocaproic acid	2-Hydroxy-3-methylvaleric acid
All	<2	<2	<2	<2	<2	<2

■ Urine (mmol/mol Creatinine)/Plasma or Serum Organic Acids (Gas Chromatography/Mass Spectrometry, GC/MS)

Age	Urine Isovaleryl-glycine	Plasma or serum iso-valeric acid (GC or GCMS)	Urine 3-OH-isovaleric acid	Urine 3-methylcrotonyl-glycine	Urine 3-methylglutaconic acid	Urine 3-methylglutaric acid	Urine 3-OH-3-methylglutaric acid
All	0–10	<10 $\mu\text{mol/l}$	0–50	0–2	0–9	0–7	0–36

6.6 Pathological Values/Differential Diagnosis

■ Urine Spot/Screening Tests

Disorder	2,4-Dinitrophenylhydrazine (DNPH) test	Ferric chloride test	Newborn screening
Maple syrup disease (MSD)	Yellow precipitate	Greenish-gray color	Leucine >2 mg/dl ^a (>153 $\mu\text{mol/l}$)

^a A patient with asymptomatic isolated 3-methylcrotonyl-CoA carboxylase deficiency was also detected via this methodology with elevated leucine.

■ Plasma Quantitative Amino Acids ($\mu\text{mol/l}$)
(Ion Exchange Column Chromatography or High-Performance Liquid Chromatography, HPLC)

6.1 MSD, presentation	Valine	Isoleucine	Leucine	Alloisoleucine	% Normal activity of BCKD ^a complex
a. Classical	496–1846	199–1298	518–5091	72–310	Less than 2
b. Intermediate	to 1000	to 1000	400–2000	Present	2–20
c. Intermittent ^b	to 1000	to 1000	50–4000	Present	2–40
d. Thiamin-responsive	to 1000	to 1000	50–5000	Present	20–40

^a BCKD, Branched-chain α -ketoacid dehydrogenase.

^b May only be abnormal during acute episodes of ketoacidosis in the intermittent form.

■ Urine Organic Acids (mmol/mol Creatinine)
(Gas Chromatography/Mass Spectrometry, GC/MS)

Disorder	2-Oxoisocaproic acid	2-Oxo-3-methylvaleric acid	2-Oxoisovaleric acid	2-Hydroxyisovaleric acid	2-Hydroxyisocaproic acid	2-Hydroxy-3-methylvaleric acid
6.1 MSD	400–4400	500–2500	300–800	850–3600	3–80	60–400

■ Urine (mmol/mol Creatinine)/Plasma or Serum Organic Acids
(Gas Chromatography/Mass Spectrometry, GC/MS)

Disorder (all ages)	Urine iso-valeryl-glycine (GC or GCMS)	Plasma or serum isovaleric acid	Urine 3-OH-isovaleric acid	Urine 3-methylcrotonyl-glycine	Urine 3-methylglutaconic acid	Urine 3-methylglutaric acid	Urine 3-OH-3-methylglutaric acid
6.2 Isovaleric acidemia	290–4980 (with episodes); 1000–3000 (between episodes)	600–5000 $\mu\text{mol/L}$ (with episodes); 10–50 $\mu\text{mol/L}$ (between episodes)	110–2000	–	–	–	–
6.3 Isolated 3-methylcrotonyl-CoA carboxylase deficiency	–	–	96–8850	40–4042	–	–	–
6.4 3-Methylglutaconic aciduria, type I (3-methylglutaconyl-CoA hydratase deficiency)	–	–	47–3840	–	168–1153	4.5–9.0	–
6.5 Barth syndrome, 3-methylglutaconic aciduria, type II (hydratase, normal)	–	–	–	–	18–140 (combined with 3-methylglutaric acid)	–	–
6.6 Costeff optic atrophy syndrome, 3-methylglutaconic aciduria, type III (hydratase, normal)	–	–	–	–	9–187 (combined with 3-methylglutaric acid)	–	–
6.7 3-Methylglutaconic aciduria, idiopathic (type IV)	–	–	–	–	23–1793	5–60	–
6.8 3-OH-3-methylglutaric aciduria (3-OH-3-methylglutaryl-CoA lyase deficiency)	–	–	60–9600	0–400	140–24200	14–3000	200–11000

6.7 Loading Tests

Loading testing is unnecessary in 6.1 through 6.8 inclusive as the diagnosis can be readily established without doing so. In 6.1, protein loading may lead to episodic metabolic decompensation. In 6.5–6.7, leucine loading is of little diagnostic relevance because enzymatic data has shown that the primary defect is not on the L-leucine degradative pathway.

6.8 Diagnostic Flow Chart

A positive Guthrie test for leucine should be repeated and confirmed by quantitative analysis. For most cases, the correct differential diagnosis depends on the quantitative analysis of urinary organic acids by combined gas chromatography/mass spectrometry (GCMS) (Fig. 6.2). Further diagnostic information may be obtained through serum/plasma carnitine analysis, analysis of urinary acylglycines, tandem mass spectrometric analysis of plasma acylcarnitine species, and (in selected instances) intact fibroblast oxidation analyses employing L-carnitine and ^{13}C -labelled leucine with acylcarnitine analysis via tandem mass spectrometry. The detection of unusual body odor (maple syrup, sweaty feet, acrid odor), acidosis, ketosis, hypoglycemia, or carnitine deficiency suggests that urine organic acid analysis should be performed. On the other hand, some patients (isolated 3-methylcrotonyl-CoA carboxylase deficiency and the 3-methylglutaconic acidurias) manifest none of these metabolic features, and urinary organic acid analysis is requested based primarily upon clinical findings (CNS or peripheral organ abnormalities, or a Reye-like disease). Moreover, patients with isovaleric acidemia are not always noted to have an “acrid” odor to their urine. Urinary organic acid profiling is essential for the correct differential diagnosis. For the interpretation of quantitative urine organic acids, see Appendix E.

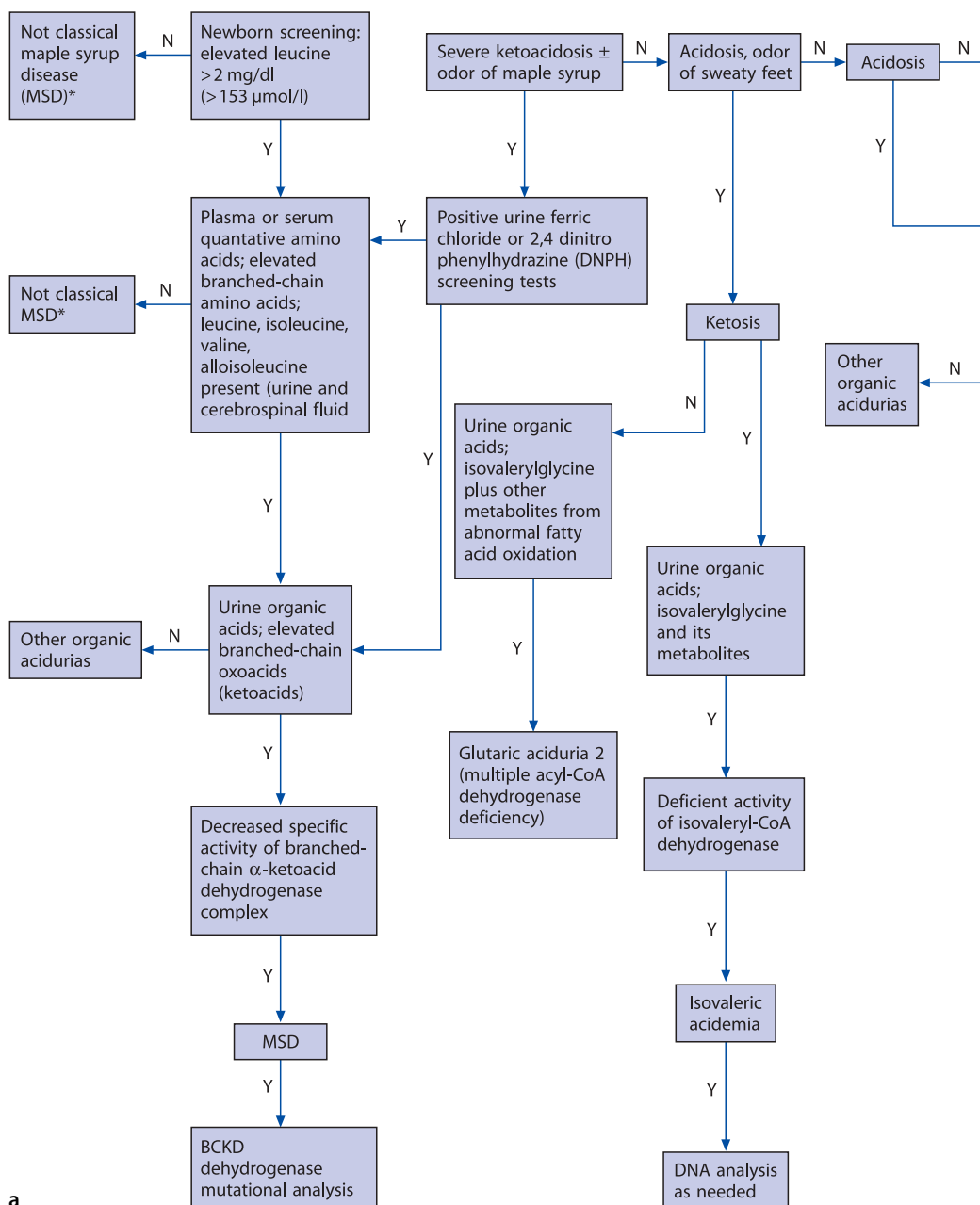


Fig. 6.2. Screening policy and the diagnostic flow chart in the differentiation of defects of L-leucine catabolism.

* Intermittent, intermediate and thiamin-responsive forms of MSD usually will not be detected by newborn screening

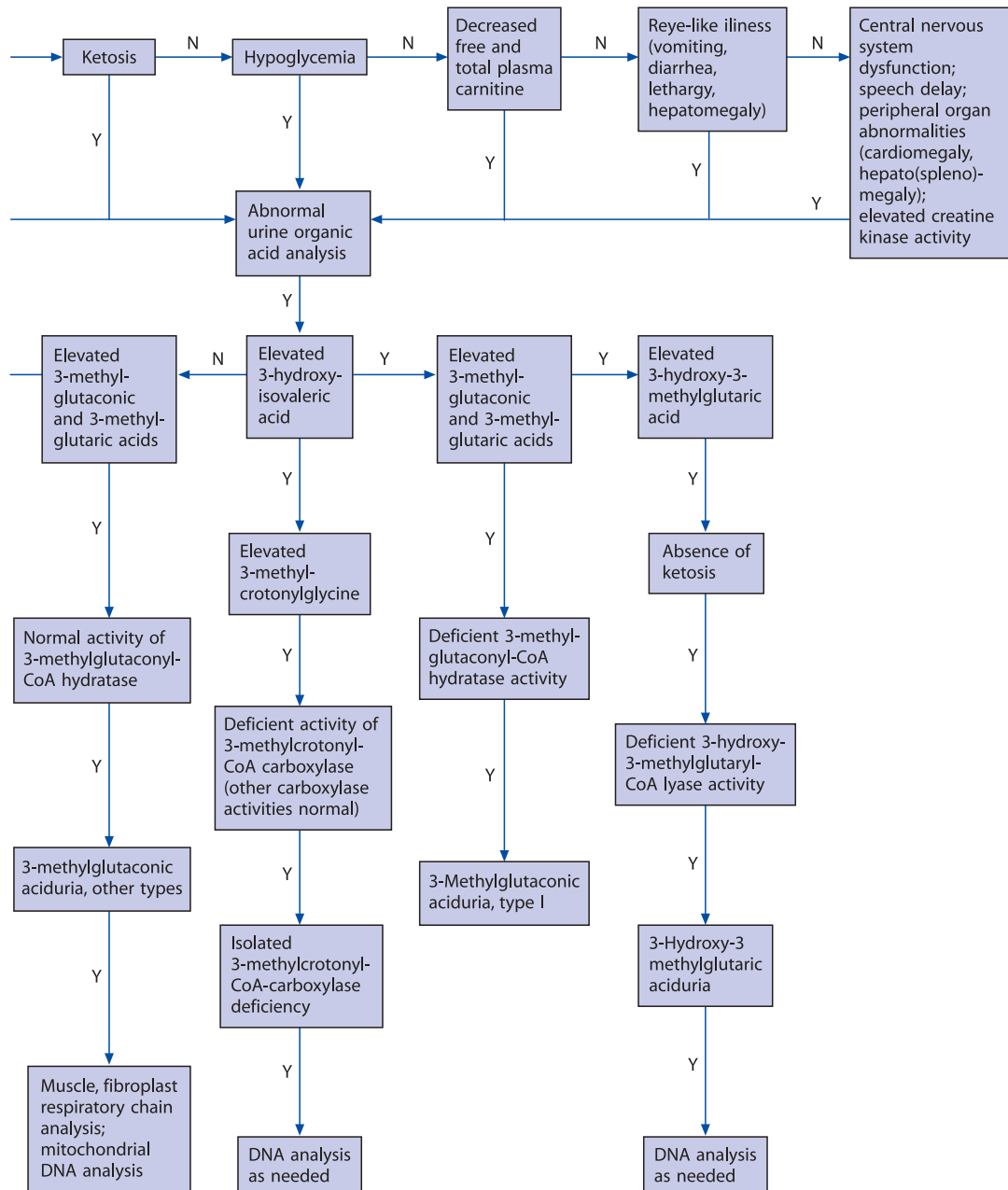


Fig. 6.2b

6.9 Specimen Collection

Disorder	Test	Preconditions	Material	Handling	Pitfalls
6.1	2,4-Dinitrophenylhydrazine (DNPH) test (positive: formation of a yellow precipitate)	None	Fresh or frozen random (U)	Keep frozen (–20 °C) until analyzed	Screening testing only. May not be positive in all patients. Usually positive if blood leucine is greater than 800 µmol/l. May be positive in other conditions with oxoacids. False positives with mandelamine and radiopaque contrast material
6.1	Ferric chloride test (positive: greenish-gray color)	None	Fresh or frozen random (U)	Keep frozen (–20 °C) until analyzed	Screening testing only. May not be positive in all patients or between acute episodes. May be positive in other conditions with oxoacids. False positives with phenylthiazines, isoniazid, acetaminophen and other medications
6.2–6.8	Carnitine (P, S)	None	Frozen (P or S)	Keep frozen (–20 °C) until analyzed	None, except lab error
6.1–6.3	Quantitative amino acids	None	Frozen (P or S)	Keep frozen (–20 °C) until analyzed	None, except lab error
6.2	Volatile short chain organic acids (GC or GC/MS)	None	Frozen (P)	Keep frozen (–20 °C) until analyzed	None, except lab error
6.1–6.8	Organic acids (GCMS)	None	Frozen random (U)	Keep frozen (–20 °C) until analyzed	None, except lab error
6.2–6.4, 6.8	Acylglycines (GCMS) (if necessary)	None	Frozen random (U)	Keep frozen (–20 °C) until analyzed	None, except lab error
6.2–6.8	Acylcarnitine fractionation (tandem MS) (if necessary)	None	Frozen (P)	Keep frozen (–20 °C) until analyzed	None, except lab error

^a For 6.8, incomplete derivatization of 3-OH-3-methylglutaric acid has been observed.

6.10 Prenatal Diagnosis

Disorder	Material	Timing, trimester
6.1 Maple syrup disease	Molecular analysis, CV sampling, cultured AFC	I, II
6.2 Isovaleric acidemia	AF, cultured AFC, CV tissue	I, II
6.3 Isolated 3-methylcrotonyl-CoA carboxylase deficiency	AF, cultured AFC, CV tissue	I, II
6.4 3-Methylglutaconic aciduria type I (3-methylglutaconyl-CoA hydratase deficiency)	AF, cultured AFC	II
6.5–6.7 3-Methylglutaconic aciduria (Barth syndrome) (3-methylglutaconyl-CoA hydratase, normal activity)	AF ^a , cultured AFC (Barth syndrome), CV tissue (Barth syndrome)	I, II
6.8 3-OH-3-methylglutaric aciduria (3-OH-3-methylglutaryl-CoA lyase deficiency)	AF, cultured AFC, CV tissue	I, II

^a Thus far, studies have been limited to patients categorized as idiopathic 3-methylglutaconic aciduria.

6.11 DNA Analysis

Disorder	Material	Methodology
6.1 Maple syrup disease	F, WBC	^a RT-PCR; genomic amplification and sequencing
6.2 Isovaleric acidemia	F, WBC	RT-PCR; genomic amplification and sequencing
6.3 3-Methylcrotonyl-CoA carboxylase deficiency	F, WBC	RT-PCR; genomic amplification and sequencing
6.5 Barth syndrome (3-methylglutaconic aciduria, hydratase normal)	WBC, lymphoblasts	RT-PCR; genomic amplification and sequencing
6.8 HMG-CoA lyase deficiency	F, WBC	RT-PCR; genomic amplification and sequencing; ASO; SSCP

^a RT-PCR, reverse transcription-polymerase chain reaction; ASO, allele-specific oligonucleotide hybridization; SSCP, single-stranded conformational polymorphism analysis.

6.12 Initial Treatment

■ General Intervention

1. Cardiorespiratory monitoring and support. Use 5 g/dl (5%) dextrose in normal saline or Ringers solution to correct hypovolemia, establish normal perfusion and urine output. Correct acidemia with intravenous NaHCO_3 . Cardiomyopathy and congestive heart failure may be particularly problematic in 6.5.
2. Use glucose and electrolyte solutions with physiological concentrations of NaCl. In 6.1, cerebral edema is sensitive to *decreases* in serum sodium and serum osmolality and sodium losses in urine may be unusually high. Use hypertonic saline and mannitol as needed to maintain serum Na >140 mEq/l and serum osmolality >290 mosm/l.
3. Hypoglycemia should be corrected rapidly with intravenous glucose 0.5–1 g/kg followed by infusion of glucose 10–12 mg/kg per minute. Give insulin 0.05–0.1 unit/kg per hour as needed to prevent serum glucose concentrations >150 mg/dl.
4. Suppress endogenous protein catabolism by enteral and/or intravenous caloric intakes >100 cal/kg per day. In disorders 6.1–6.5, give 30–50% of calories as lipid. In 6.6, lipid and several ketogenic amino acids are sources of HMG acid and should also be restricted.
5. Support endogenous protein synthesis with enteral or intravenous amino acid mixtures devoid of leucine. Supplement as needed to prevent isoleucine and valine deficiencies, including in maple syrup disease, wherein isoleucine and valine rapidly become depleted and 40–80 mg/kg per day of each of these two essential amino acids are needed to support high rates of protein synthesis and maximum rates of leucine decrease.
6. In 6.2, intracranial hemorrhages may occur in association with thrombocytopenia. Monitor platelet counts and clotting times. Transfuse with platelets and give vitamin K as necessary to decrease the risk of hemorrhages.

■ Specific Intervention (by Disorder)

6.1 Thiamin pharmacologic doses 100–500 mg/day. The Mennonite variant is nonresponsive. Thiamin, lipoic acid and L-carnitine, in theory, may help maintain activity at the pyruvate dehydrogenase and α -ketoglutaric acid dehydrogenase complexes.

6.1, 6.2 Enteral glycine supplementation 250 mg/kg per day.

6.2, 6.3, 6.4, 6.6 All involved enzymes complex with CoA. Supplementation with L-carnitine (100 mg/kg per 24 hours) and pantothenic acid (25–50 mg per 24 hours) may have therapeutic value.

6.13 Summary and Comments

The encephalopathy and coma caused by disorders of leucine metabolism are associated with lasting brain injury and death. Diagnostic tests such as amino acid quantification, urine organic analysis, and acylcarnitine analyses must be done emergently. The initial interventions described above and definitive therapies also should be undertaken with urgency. Fortunately, increasingly, disorders of leucine metabolism are diagnosed through newborn screening, and many infants are symptomatic when the diagnosis is made. Initial medical interventions are less complex and outcomes can be expected to be better.

Disorders of leucine metabolism present with a complex array of clinical and laboratory findings. The odors associated with MSD and isovaleric acidemia are distinctive and are virtually pathognomonic in infants who have ketonuria and are encephalopathic. Ketonuria in a neonate, seen with several disorders in the leucine pathway, should always be considered a sign of an underlying metabolic disorder and is an indication for urgent amino acid quantification, urine organic analysis, and acylcarnitine analyses. Non-ketotic hypoglycemia is currently more often thought of in association with disorders of fatty acid oxidation, but HMG-CoA lyase deficiency must also be ruled out by appropriate studies. Children older than 10–15 years and adults are always diagnostic problems. Patients with 3-methylcrotonylglycinuria (6.3) may be seen by a neurologist with a complaint of muscle weakness. Patients with Barth's form of 3-methylglutaconic aciduria may have been followed by cardiologists and hematologists for many years and quantitative urine organic acids may not have been done. As with all metabolic disorders, the classical neonatal presentation of any one defect of leucine degradation represents only one extreme of the disorder. Timely recognition and treatment of infants and children with the full ranges of clinical and biochemical problems requires a high index of suspicion, a low threshold for sending quantitative biochemical tests, and expanded neonatal screening.

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