



Methylmalonic Acidemia

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ABSTRACT

Methylmalonic acidemia is a rare autosomal-recessive inborn error of metabolism which is caused by a downstream defect in the propionate metabolic pathway. Methylmalonyl-CoA mutase, the enzyme which converts methylmalonyl-CoA to succinyl-CoA is found to be non-functional in these disorders. The metabolic block in these disorders often leads to severe keto- and organic acidosis, hyperammonemia, hyperglycinemia, and hypoglycemia with marked accumulation of methylmalonate in body fluids and tissues of infants and children. The clinical presentation and pathology is accordingly severe in these disorders: Widespread psychomotor dysfunction, failure to thrive, dystonia, and hepatomegaly are some of the symptoms and the symptoms can extend to long-term neurological and systemic impairment. Hematological abnormalities are also observed, especially in cases where methylmalonic acidemia is accompanied by homocystineuria. The disorder is lethal in its early (neonatal) onset form, and may also manifest as a chronic problem, with episodes of acute decompensation and, in rare cases, the patient may be completely asymptomatic ('benign' form). In this article, a comprehensive survey of present literature is presented with an emphasis on the molecular basis of the disease.¹⁻³ Current methods of diagnosis, treatment, and the prognosis for these patients are discussed and some current areas of investigation explored.

INTRODUCTION

Propionyl-CoA, its parent metabolic compounds, and molecules derived from it (e.g., methylmalonyl-CoA) are important precursors of succinyl-CoA, an important Krebs cycle intermediate. Recently, this metabolic pathway came to the attention of clinicians struggling with disturbing diseases. Researchers reported that adenosylcobalamin (AdoCbl), a metabolic derivative of cobalamin (Cbl; vitamin B₁₂), is an essential coenzyme in the final conversion of L-methylmalonyl CoA to succinyl-CoA.⁴⁻⁶ Patients with acquired Cbl deficiency excreted large amounts of methylmalonic acid in urine.⁷⁻¹⁰ Subsequently, infants not deficient in Cbl were described with similar signs.^{11,12} The absence of methylmalonic acid in previously described ketotic hyperglycinemia¹³ suggested a novel disorder which was named methylmalonic acidemia. The former was later ascribed to a defect in propionate metabolism, and named

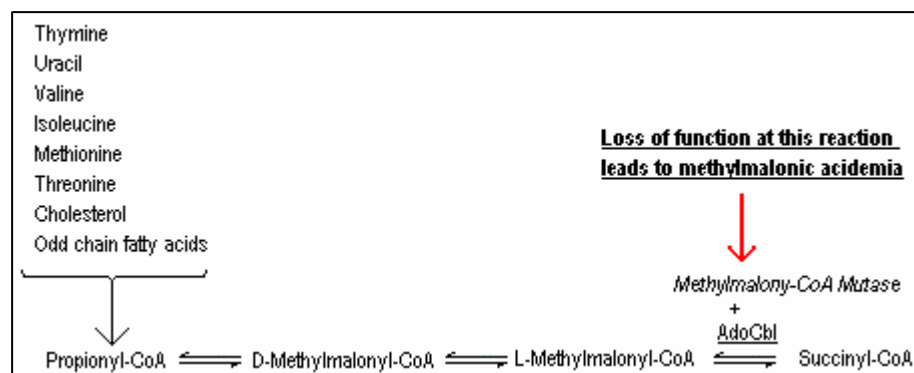


Figure 1. This schematic diagram illustrates the precursors and products of propionyl-CoA metabolism.^{26,27} Propionyl-CoA is converted first to D-methylmalonyl-CoA,²⁸⁻³⁰ and then L-methylmalonyl-CoA is produced via racemization.³¹ L-methylmalonyl-CoA is converted to succinyl-CoA by methylmalonyl-CoA mutase via a mechanism involving the adenosylcobalamin (AdoCbl) coenzyme.²⁵ A deficiency of methylmalonyl-CoA mutase leads to methylmalonic acidemia.

propionic acidemia.^{14,15}

Methylmalonic acidemia and propionic acidemia are now recognized as the most common inborn errors of organic acid metabolism.

In addition, the Cbl deficient cases of

methylmalonic acidemia who responded to Cbl supplements^{16,17} had a defect in AdoCbl¹⁸⁻²²

synthesis, the coenzyme of *methylmalonyl-CoA mutase*,⁴⁻⁶ the enzyme catalyzing the last step of

the propionic acid pathway (Figure 1).²³⁻²⁵ Also, methylmalonic acidemia patients were described with homocystinuria and hypomethioninemia, who also had *methionine synthase*³²

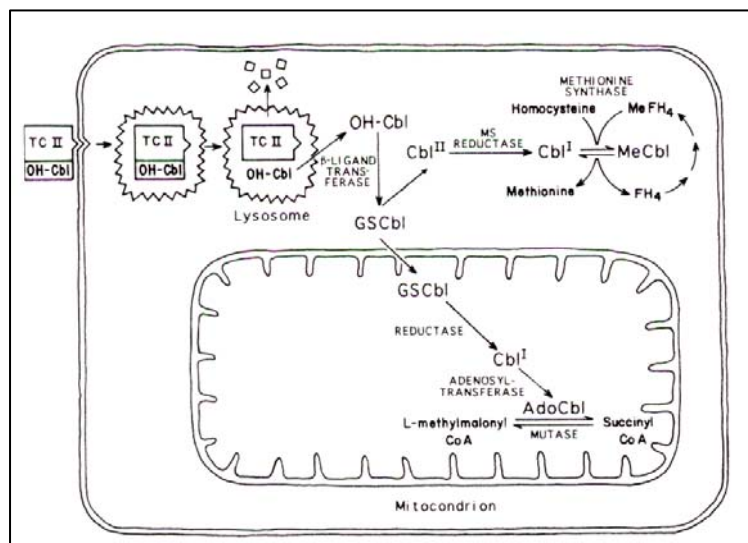


Figure 2. Pathway of cobalamin (vitamin B₁₂; Cbl) uptake. Dietary Cbl is endocytosed into a target cell as the TC II/OH-Cbl complex.³⁶ The free Cbl is released into the cytosol after lysosomal processing. From there it is reduced to either the MeCbl or AdoCbl coenzymes. [TC II = transcobalamin II; OH-Cbl = hydroxocobalamin; GSCbl = glutathionylcobalamin; MeCbl = methylcobalamin; MeFH₄ = methyltetrahydrofolate; AdoCbl = adenosylcobalamin; Cbl^{III}, Cbl^{II}, and Cbl^I = cobalamins with cobalt valence of 3⁺, 2⁺, and 1⁺, respectively.] Figure adapted from Fenton, WA, Gravel, RA, & Rosenblatt, DS. "Disorders of Propionate and Methylmalonate Metabolism," in Scriver, CR, *et al.* (editors) *The metabolic and molecular basis of inherited disease*. Volume II, 2001, p 2165.¹ Copyright © The McGraw-Hill Companies, Inc., New York, 2001.

deficiency owing to defective synthesis of methylcobalamin (MeCbl)³³⁻³⁵—the Cbl derived coenzyme of methionine synthase, a cytosolic enzyme. Figure 2 to describes the current understanding of cobalamin metabolism in cells.

The study of methylmalonyl-CoA mutase enzyme has advanced significantly. It belongs to the group of enzymes catalyzing unusual 1,2-rearrangements and is the only member of this class found in both bacteria and animals.³⁷ Ledley, *et*

*al.*³⁸ localized the methylmalonyl-CoA mutase (*mcm*) gene to the short arm of human chromosome 6. Later the *mcm* gene was found to span >35 kilobases (comprising 13 exons).³⁹ Jansen and Ledley⁴⁰ found the coding sequence of one subunit to be 718 amino acids in length (≈80 kilodaltons) with a 32 amino acid mitochondrial targeting sequence.^{40,41} Mancina, *etal.*^{37,42} solved the protein structure of a homologue of the MCM protein using X-ray diffraction.

ANALYSIS OF DISEASE CONDITION AND DEFECTS

The current body of data demonstrates that there are 7 distinct molecular defects in methylmalonate metabolism. Two of them, mut^0 and mut^- , are a direct result of defect in the mutase apoenzyme: mut^0 results from complete deficiency; and mut^- results from partial deficiency of methylmalonyl-CoA mutase. The other 5 defects are the *cbl* defects, so-called because they result from impaired cobalamin metabolism. Two are distinct defects of AdoCbl synthesis (*cblA* and *cblB*), and the three others are complicated by MeCbl deficiency in addition to AdoCbl (*cblC*, *cblD*, and *cblF*). The defects leading to isolated mutase deficiency (mut^0 , mut^- , *cblA*, & *cblB*) and those leading to combined AdoCbl and MeCbl deficiency (*cbl C, D, & F*) can be treated as separate groups.

Isolated mutase deficiency

Clinical and laboratory presentation

More than 100 children with isolated mutase deficiency have been documented.¹ The four known etiologies which result in such patients present with similar clinical findings. Matsui, *et al.*⁴³ surveyed the natural histories of 45 patients with these etiologies: 15 mut^0 , 5 mut^- , 14 *cblA*, and 11 *cblB*. Equal numbers of males and females were in each group. The most common signs and symptoms at the onset of clinical difficulty (usually brought about in children by an infection or excessive protein intake²) are listed in **Table 1** and were similar in all etiologies. Patients in mut^0 class presented earlier than those in other groups. Whereas 80 percent of children in the mut^0 class became ill in the first week of life, less than half the children in the three other groups were ill during this interval.⁴³ Furthermore, clinical onset occurred in 90 percent of the mut^0 patients before the end of the first month, whereas onset beyond the first

month was observed in an appreciable fraction of patients the other three groups. Another survey reached similar conclusions.⁴⁴

Signs and Symptoms at Onset	Mutant Class				
	<i>cblA</i>	<i>cblB</i>	<i>mut⁻</i>	<i>mut⁰</i>	Total
Lethargy	78	83	100	85	84
Failure to thrive	75	86	40	77	73
Recurrent vomiting	58	86	80	77	73
Dehydration	64	86	100	62	71
Respiratory distress	89	67	50	55	67
Muscular hypotonia	44	57	33	91	63
Developmental retardation	36	33	25	65	47
Hepatomegaly	11	67	0	57	41
Coma	50	29	40	38	40

Numerical values represent percentage of patients in each group.

Adapted from Matsui, *et al.*⁴⁵ Copyright © 1983 Massachusetts Medical Society. All rights reserved.

The laboratory findings in affected patients also show marked similarity between the etiologies. Serum Cbl concentrations were routinely normal. The findings are listed in **Table 2**. Earlier case reports⁴⁵ indicated that hypoglycemia, a parameter not assessed in this survey,¹ occurs in about 40% of patients.

Findings at Clinical Onset	Mutant Class				
	<i>cblA</i>	<i>cblB</i>	<i>mut⁻</i>	<i>mut⁰</i>	Total
Normal serum cobalamin	100	100	100	100	100
Metabolic acidosis	100	88	100	85	92
Ketonemia/ketonuria	78	67	100	85	81
Hyperammonemia	50	83	80	75	71
Hyperglycinemia/glycinuria	70	83	40	70	68
Leukopenia	70	45	60	62	60
Anemia	10	45	0	58	55
Thrombocytopenia	75	45	40	40	50

Numerical values represent percentage of patients in each group.

Adapted from Matsui, *et al.*⁴⁵ Copyright © 1983 Massachusetts Medical Society. All rights reserved.

A number of pathologic signs involving various organ systems have been documented and characterized to some degree. These are presented in **Table 3**.

TABLE 3: PATHOLOGIC SIGNS OF METHYLMALONIC ACIDEMIA
<i>Metabolic stroke</i> (following episodes of metabolic decompensation) ⁴⁶⁻⁴⁸
<i>Pancytopenia</i> ^A (about half the reported patients) ⁴³
<i>Neutropenia</i> ^A and <i>thrombocytopenia</i> ^A (during the first year) ³
<i>Anemia</i> ^A (neonatal period) ³
Susceptibility to <i>viral</i> ^A and <i>bacterial</i> ^A infections ⁴⁹
<i>Chronic renal insufficiency</i> ⁵⁰⁻⁵² (May be associated with <i>renal tubular acidosis</i> and <i>chronic tubulointerstitial nephritis</i> ⁵³⁻⁵⁶)
<i>Hepatic steatosis</i> ⁵⁰⁻⁵⁶
<i>Ataxia</i> and <i>mental retardation</i> possibly resulting from <i>demyelination of subcortical and other neurons</i> and <i>characteristic lesions in the globus pallidus</i> (evident from MR and CT imaging) ^{B,46,57-59}
<i>Failure of linear growth</i> (seen in some <i>short and obese patients</i>) ^{2,60}
<i>Acute pancreatitis</i> (due to metabolic decompensation) ³
^A These signs may be due to methylmalonate inhibition of marrow stem cell growth in a concentration-dependent fashion ⁶¹
^B Other neurologic consequences have also been observed ^{62,63} including some in animal models. ⁶⁴⁻⁶⁶ Some may be due to excessive methylmalonate levels ^{58, 64-66} or the effects of hyperammonemia ⁶⁷

Mutase deficiency may sometimes be asymptomatic.⁶⁸ Presumably, these patients have an enzyme defect which retains just enough activity that homeostasis is maintained.¹ Another report describes patients with methylmalonic aciduria urine levels of approximately 1400 mmol/mol creatinine, who had normal somatic and cognitive outcomes.⁶⁹ Levy, *et al.*⁷ followed closely a child who suffered several acute episodes in childhood but afterwards remained asymptomatic, with an outstanding academic performance. Interestingly, one study reports that children with methylmalonic acidemia have an increased resting energy expenditure (REE) in spite of being asymptomatic.⁷⁰

On the otherhand, other groups appear to have a puzzlingly mild methylmalonic acidemia without demonstrable defect in methylmalonyl-CoA mutase activity or in Cbl metabolism.^{71,72} The patients in at least one report⁷² presented with psychomotor delay, no metabolic acidosis and methylmalonic semialdehyde dehydrogenase deficiency.

The most prominent chemical abnormality observed in patients with the isolated mutase deficiency is large amounts of methylmalonic acid in urine and blood, as indicated in **Table 4**.^{7,45}

TABLE 4: METABOLIC DERANGEMENTS OF METHYLMALONIC ACID LEVELS IN CHILDREN WITH METHYLMALONIC ACIDEMIA (ISOLATED ENZYME DEFICIENCY TYPE).		
<i>Quantity</i>	<i>Normal Subjects</i>	<i>Methylmalonic Acidemia Patients</i>
methylmalonate excreted daily	<0.04 mmole (5 mg)	2.1 to 49 mmoles (240 to 5700 mg) in a 24-h period. ⁷
Plasma & CSF ⁴⁵ methylmalonate concentrations	<i>Undetectable</i>	0.22 to 2.9 mM (2.6-34 mg/dl). ⁷

Importantly patients with mild, late-onset, or “benign”⁶⁸ disease may have much lower levels of methylmalonate, particularly when clinically asymptomatic.^{8,68} Propionate and some of its upstream precursors also accumulate in blood and urine of these patients,^{14, 73-76} thus accounting for the great similarity observed in the presentation of propionic and methylmalonic acidemias.^{2,77}

Lastly, research has shown that administration of protein and amino acid precursors of propionate (and methylmalonate), such as methionine, threonine, valine, and isoleucine, augments methylmalonate accumulation and, in some instances, ketosis or acidosis.^{11,12,14,16} When Cbl-responsive patients are given supplements vitamin B₁₂, such augmentation by methylmalonate precursors is lessened.⁷⁸

Physiologic disturbances in isolated mutase deficiency (*mut*⁰, *mut*⁻, *cblA*, & *cblB*)

All studies *in vivo* and *in vitro* in patients with methylmalonic acidemia indicate that the primary block in the conversion of methylmalonyl-CoA to succinyl-CoA explains the methylmalonate accumulation and accompanying biochemical changes.¹ However, primary block does not explain several important physiologic disturbances such as acidosis, hypoglycemia, hyperglycinemia, and hyperammonemia. Oberholzer, *et al.*¹¹ suggested an explanation for the observed acidosis that methylmalonyl-CoA might be “trapping” the cellular supply of coenzyme A, leading to impaired carbohydrate metabolism. Alternatively,

methylmalonyl-CoA might interfere with gluconeogenesis,⁷⁹ leading directly to hypoglycemia, and the subsequent increase in lipid catabolism could cause ketoacidosis. Halperin, *et al.*⁸⁰ showed that methylmalonate inhibited the transmitochondrial shuttle of malate and argued that impairment of this key step in gluconeogenesis could lead to hypoglycemia. Treacy, *et al.*⁸¹ have suggested that a deficiency of glutathione may also contribute to lactic acidosis in these patients.

Additionally, in methylmalonic acidemia the accumulated organic acids or their CoA esters inhibit intramitochondrial glycine cleavage and an enzyme associated with the urea cycle.⁸²⁻⁸⁸ These are probable causes of hyperglycinemia and hyperammonemia in affected children. Carnitine deficiency results from decreased renal handling of filtered carnitine and the excretion of acylcarnitine derivatives formed from organic acids.^{2,89,90} This deficiency may contribute to muscle hypotonia and other clinical findings (Tables 1 & 3).

Inheritance pattern and epidemiology (*mut*⁰, *mut*⁻, *cblA* & *cblB*)

Each of the four etiologies for isolated methylmalonyl-CoA mutase deficiency are inherited as autosomal-recessive traits, as a number of studies demonstrate.^{40,43,91-98}

The prevalence of methylmalonic acidemia is difficult to define precisely. One survey in Massachusetts suggested an occurrence of 1:48,000 infants,⁹⁹ while another in Quebec suggested 1:61,000 infants.¹⁰⁰ Others have suggested a figure of 1:29,000.^{99,68} A much greater prevalence of between 1:1,000 and 1:2,000 has been reported in Middle Eastern populations.⁶⁰

Molecular characterization of isolated mutase deficiency (*mut*⁰, *mut*⁻, *cblA*, & *cblB* defects)

Evidence for defect in methylmalonyl-CoA mutase apoenzyme came from *in vitro* studies showing instances where mutase enzyme activity could not be restored at saturating AdoCbl concentrations, whereas, in other cases, the activity was restored to normal.^{101,102} Subsequently, much has been learned about the *mut*⁰ and *mut*⁻ defects.

The mut^0 defect, constituting two-thirds of the mut group, shows mutase activity which is undetectable in cultured fibroblasts (<0.1% of control), even in the presence of excess AdoCbl.^{96,102} The molecular flaws in mut^0 patients range from no enzyme synthesis at all, to unstable and rapidly degraded enzyme, to highly reduced enzyme levels—and a problem with mitochondrial targeting in one case.¹⁰³⁻¹⁰⁵

The other defect, mut^- , involves a structurally abnormal mutase apoenzyme. The mutated enzymes in extracts from these cells retain 2 to 75% of normal activity, bind AdoCbl 200 to 5000 times less well than normal enzyme, and exhibit increased thermolability.^{96,106,108} Since individuals who appear to be mut^0/mut^- compound heterozygotes are affected, both defects must

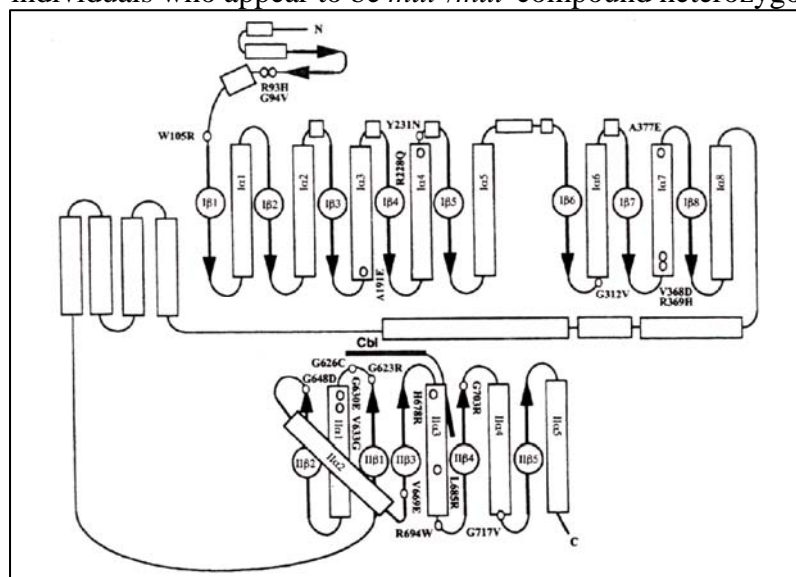


Figure 3.¹⁰⁸ Schematic representation of the secondary structure of the methylmalonyl CoA mutase enzyme. The adenosylcobalamin (AdoCbl) binding site is indicated. The locations of a number of identified mutations are indicated (see Text & Table 3). From Thoma, NH; Leadlaey, PF: Homology modeling of human methylmalonyl-CoA mutase: A structural basis for point mutations causing methylmalonic aciduria. *Protein Sci* 5: 1922, 1996.¹⁰⁸ Copyright © 1996, Cold Spring Harbor Laboratory Press.

reflect abnormalities of the same locus (i.e., the mut gene).^{96,106}

Moreover, considerable information exists on the molecular abnormalities underlying the mut group of defects in methylmalonic acidemia. Ledley, *et al.*⁹⁸

initially found reduced mRNA levels in some lines. **Table 5** lists many of the mutations identified

to date. To date, about 34 mutations and 2 benign sequence changes have been

identified.^{40,91,92,105,109-121} A number of mutations have been characterized which are common

among people from different racial or ethnic groups.^{111,113,116,117} Figure 3 shows a linear

representation of the structure of human mutase,¹⁰⁸ based on the crystal structure of a bacterial homologue.^{37,42} On it are indicated the locations of a number of the missense mutations in mutase identified so far. The effects of some of these have been rationalized in terms of the predicted three dimensional structure.^{92,108,110,118,122}

TABLE 5. MUTATIONS IN METHYLMALONIC ACIDEMIA. (PHENOTYPE CONFIRMED BY GENE TRANSFER WHERE INDICATED.)				
Amino Acid	Base	Exon	Phenotype	Investigators
Q18X	C→T, 128	II	<i>mut</i> ⁰	Ledley, <i>et al.</i> 1990
R93H	G→A, 354	II	<i>mut</i> ^{0a}	Raff, <i>et al.</i> 1991
W105R	T→C, 389	II	<i>mut</i> ⁰	Jansen and Ledley, 1990
A377E	C→A, 1206	VI	<i>mut</i> ⁰	Jansen and Ledley, 1990
G623R	G→A, 1943	XI	<i>mut</i> ⁰	Qureshi, <i>et al.</i> 1994 ¹⁰⁹
G626C	G→T, 1952	XI	<i>mut</i> ⁰	Crane and Ledley, 1994 ¹¹⁰
G630E	G→A, 1965	XI	<i>mut</i> ⁰	Crane and Ledley, 1994 ¹¹⁰
G648D	G→A, 2019	XI	<i>mut</i> ⁰	Crane and Ledley, 1994 ¹¹⁰
R694W	C→T, 2156	XII	<i>mut</i> ⁰	Crane and Ledley, 1994 ¹¹⁰
G703R	G→C, 2183	XII	<i>mut</i> ⁰	Qureshi, <i>et al.</i> 1994 ¹⁰⁹
G717V	G→T, 2226	XIII	<i>mut</i> ⁰	Crane, <i>et al.</i> 1992 ^{111,115}
H532R	A→G, 1671	IX	Polymorphism	Crane, <i>et al.</i> 1992; ^{111,115} Qureshi, <i>et al.</i> 1994; ¹⁰⁹ Crane and Ledley, 1994 ¹¹⁰
G671I	G→A, 2087	XII	Polymorphism	Crane, <i>et al.</i> 1992; ^{111,115} Qureshi, <i>et al.</i> 1994; ¹⁰⁹ Crane and Ledley, 1994 ¹¹⁰
E84X	G→T, ^b 326			Ogasawara, <i>et al.</i> 1994i ¹¹²
A197T	G→A, 665			Ogasawara, <i>et al.</i> 1994i ¹¹²
FrSh231	769delCA ^b			Ogasawara, <i>et al.</i> 1994i,ii ^{112,113}
E117X	G→T, ^b 425			Ogasawara, <i>et al.</i> 1994i,ii ^{112,113}
Y231N	T→A, ^c 767			Kogekar, <i>et al.</i> 1997 ⁹²
V368D	T→A, 1179			Ogasawara, <i>et al.</i> 1994i ¹¹²
R369H	G→A, ^b 1182			Kogekar, <i>et al.</i> 1997; ⁹² Ogasawara, <i>et al.</i> 1994i ¹¹²
FrSh655	1952del13			Touraine, <i>et al.</i> 1995
V669E	T→A, 2082			Ogasawara, <i>et al.</i> 1994i,ii ^{112,113}
H678R	A→G, ^b 2109			Kogekar, <i>et al.</i> 1997 ⁹²
^a This mutation exhibits interallelic complementation (Raff, <i>et al.</i> 1991; Crane and Ledley, 1994; ¹¹⁰ Qureshi, <i>et al.</i> 1994 ¹⁰⁹)				
^b Mutations identified by Kogekar, <i>et al.</i> 1997 ⁹² are from <i>mut</i> ⁻ cell lines but may individually express either <i>mut</i> ⁻ or <i>mut</i> ⁰ phenotypes.				
^c Associated with reduced mRNA levels.				
Table adapted from Ledley and Rosenblatt, 1997. ¹²¹ Note: this table indicates mutations that had been identified till 1997. See text for more recent findings. ^{108,117-120}				

The molecular abnormality of adenosylcobalamin synthesis—i.e., the Cbl-responsive forms of the disease (i.e. the *cbl*- defects)—are associated with functional deficiency of specific mitochondrial enzymes of AdoCbl synthesis (Figure 2).^{23,24} In particular, two mutant classes have been differentiated among patients defective only in AdoCbl synthesis, that is, *cblA* and *cblB*.^{93-95,123} The *cblA* defect is associated with a deficiency of a mitochondrial Cbl reductase.¹²⁴ There is some evidence indicating a possibility of interallelic complementation in this defect.¹²⁵ The second defect designated *cblB*, results from deficiency of cob(I)alamin adenosyltransferase.⁹⁷

Combined methionine synthase and methylmalonyl-CoA mutase deficiency

Presentation and pathology

The defects in this category differ from isolated mutase deficiency in that they demonstrate both methylmalonic acidemia and homocystineuria. Many patients with the inherited combined disorder have been subject of individual case reports.^{126, 127-143} Cells from these children comprise three biochemically and genetically distinct complementation groups, designated *cblC*, *cblD*, and *cblF*.^{93,94,125,144} Of these, the *cblC* defect is inherited as an autosomal-recessive trait,⁹³ but the mode of inheritance of *cblD* and *cblF* defects is not yet known.

Among the more than 100 patients characterized with *cblC* defect, clinical findings have varied widely, and some cases diagnosed only in adult life. In a review of 50 patients,¹⁴⁴ 44 had onset in the first year of life and 6 had onset after 6 years of age, and 13 early-onset patients died. The clinical presentation and laboratory findings of *cblC* patients are given in **Table 6**.

Neither of the two brothers in the *cblD* group¹²⁶ had any clinical problems until the older brother presented with severe behavioral pathology and moderate mental retardation. The 2-year-old sibling was asymptomatic, although biochemically affected, and neither had any hematologic abnormalities.

Additionally, six patients have been reported in the *cbIF* group. The clinical and laboratory findings from patients in this category are presented in **Table 7**.

TABLE 6. CLINICAL PRESENTATION AND LABORATORY FINDINGS IN PATIENTS WITH <i>cbIC</i> TYPE COMBINED METHYLMALONYL COA MUTASE AND METHIONINE SYNTHASE FUNCTIONAL DEFICIENCIES	
Early-Onset ($\approx 88\%$) ¹⁴⁵	Late-Onset ($\approx 12\%$) ¹⁴⁵
Feeding difficulties	Decreased cognitive performance
Hypotonia	Confusion
Failure to thrive	Dementia
Seizures	Delirium
Microcephaly	Myelopathy
Developmental delay	Tremor
Cortical atrophy	Pigmentary retinopathy ^c
Hydrocephalus	Skin lesions ^a
Nystagmus	Megaloblastic ^b anemia
Pigmentary retinopathy	Thrombocytopenia ^b
Decreased visual acuity	Leukopenia ^b
Megaloblastic anemia	Neutropenia ^b
Thrombocytopenia	Cbl = <u>Normal</u>
Leukopenia	Folate = <u>Normal</u>
Neutropenia	
Renal failure ^d	
^a reported by Howard, <i>et al.</i> ¹⁴⁶	
^b hematologic abnormalities observed in half the later-onset patients	
^c only one case observed	
^d only a few cases observed.	

Molecular characterization of combined deficiency states (cbIC, cbID, & cbIF defects)

A variety of experiments

have revealed that in *cbIC* and *cbID* forms of the disease, the cellular metabolism of cobalamin (Cbl, vitamin B₁₂) becomes deranged such that the coenzymes for both methylmalonyl-CoA mutase and methionine synthase (AdoCbl and MeCbl, respectively) are

improperly synthesized.^{33,127} Experiments have indicated lower Cbl content in liver and kidney fibroblasts,^{33,128,148,149} and inability of cells to retain radioactively labeled CN-Cbl or convert it to either MeCbl or AdoCbl.^{24,94,150} The two enzymatic deficiencies improve with OH-Cbl supplementation of growth medium.^{24,33,94,95,151} *cbIC* is unable to convert CN-Cbl to OH-Cbl, a necessary prerequisite for MeCbl and AdoCbl synthesis, indicating a defective cytosolic cob(III)alamin reductase.^{152,153,154} This is indeed the case for both *cbIC* and *cbID*.^{155,156} Glutathionyl Cbl intermediate in the reductive pathway may also be mutated in these groups.¹⁵⁷

Although the *cb1F* defect results in impaired AdoCbl and MeCbl synthesis, affected cells accumulate unmetabolized CN-Cbl in lysosomes, indicating a deficiency in the process by which cobalamin metabolites exit from lysosomes after being taken up (Figure 2).

Clinical signs and symptoms	Laboratory findings
Small size for gestational age/Inadequate weight gain ^{140,141,147}	Methylmalonic aciduria ^{140,141}
Poor feeding ^{140,141,147}	Cbl malabsorption ¹⁴⁰⁻¹⁴²
Failure to thrive ^{142,147}	Macrocytosis ^{140,141}
Developmental delay ¹⁴²	Homocysteinuria ^{140,141}
Persistent stomatitis ¹⁴⁰⁻¹⁴²	Hypoglycemia ¹⁴³
Growth retardation ^{140,141}	Thrombocytopenia ¹⁴³
Minor facial anomalies ^{140,141}	Neutropenia ¹⁴³
Dextrocardia ^{140,141}	Anemia ¹⁴²
Persistent rash ^{140,141}	Low serum Cbl ¹⁴²
Premature arthritis ¹⁴³	
Confusion ¹⁴³	
Disorientation ¹⁴³	
Pigmentary dermatitis ¹⁴³	
Aspiration pneumonia ¹⁴³ (at birth)	
Hypotonia ¹⁴³	
Lethargy ¹⁴³	
Recurrent infections ¹⁴²	
Gastroesophageal reflux ¹⁴⁷	

Differences between pathophysiology of combined enzyme deficiency and isolated methylmalonyl-CoA mutase deficiency

In general the two groups of combined methionine synthase and methylmalonyl-CoA mutase deficiency and isolated methylmalonyl-CoA mutase share more in common than not. But important differences set these categories of methylmalonic acidemia apart which should be noted. A comparison of the two groups of disorders is presented in **Table 8**. One should also note that homocystinuria present in the combined disease form may not always be detectable although methionine synthase activity is reduced.¹⁴⁰⁻¹⁴³

<i>Combined methionine synthase and methylmalonyl-CoA mutase deficiency</i>	<i>Isolated methylmalonyl-CoA mutase deficiency</i>
<i>megaloblastic anemia</i> (in <i>cb1C</i> patients reflects deficiency of methionine synthase) ^{145,146}	no such hematologic dysfunction
Early and severe <i>central nervous system anomalies</i> ¹⁴³ (in <i>cb1C</i> group) <u>result from methionine synthase deficiency</u> ¹⁵⁸	<u>neurologic problems result from severe metabolic ketoacidosis</u> ^{46,57-59,62-66}
<u>Less severe methylmalonic aciduria</u>	<u>Severe methylmalonic aciduria</u>
<u>Hyperglycinemia not reported</u>	Hyperglycinemia <u>frequently present</u> (Table 2)
<u>Hyperammonemia not reported</u>	Hyperammonemia <u>frequently present</u> (Table 2)

DIAGNOSIS

A number of techniques exist for the diagnosis of methylmalonic acidemia and these are indicated in **Table 9**. Other sources of ketoacidosis must also be ruled out. Confirmation and etiologic designation (i.e., *mut* or *cbl* defect) depend on studies with cultured cells and extracts therefrom (see Table 9).^{159,160} Prenatal detection of methylmalonic acidemia has also been accomplished as indicated in Table 9.

TABLE 9: TECHNIQUES FOR DIAGNOSING METHYLMALONIC ACIDEMIA.
<i>calorimetric assays for urinary methylmalonate (simplest technique)</i>
<i>such as automated tandem mass spectrometry^{a,161}</i>
<i>gas-liquid chromatography (GLC)^a</i>
<i>gas chromatography—mass spectrometry (GC-MS)^{a,1,2}</i>
<i>Direct measurement of serum Cbl concentration (for excluding Cbl deficiency)</i>
<i>For confirmation and etiologic designation (i.e., <i>mut</i> or <i>cbl</i> defect):¹</i>
<i>Studies of Cbl uptake and AdoCbl formation by intact cultured fibroblasts</i>
<i>Assays of mutase activity in cell extracts</i>
<i>Genetic complementation studies with cultured cell heterokaryons</i>
Prenatal detection of methylmalonic acidemia:
<i>Measurement of methylmalonate in amniotic fluid and maternal urine at mid-trimester^{162,163}</i>
<i>Studies of mutase activity and Cbl metabolism in cultured amniotic fluid cells.^{93,163,164}</i>
<i>Assays of [¹⁴C]propionate utilization^{165,166} uncultured chorionic villus biopsy specimens (Proven unsatisfactory?¹⁶⁷)</i>
^a Assays for serum and urinary methylmalonate.

Based on current understanding, the presence of methylmalonic aciduria, homocystinuria, and normal serum Cbl concentrations is the combination needed to distinguish patients in the combined deficiency groups from those with isolated mutase deficiency, and one of several other causes of homocystineuria. Such distinctions can be confirmed by cell studies. Thus, patients in this category can be expected to present with a combination of symptoms from both the isolated mutase deficiency (Tables 1-3) and those attributable to the methionine synthase deficiency (Tables 6-7).^{146,158}

TREATMENT

The acute management of methylmalonic acidemia^{2,60} involves (1) protein elimination with provision of adequate calories to suppress gluconeogenesis using intravenous fluids with glucose, (2) administration of intravenous bicarbonate to correct acidosis, and (3) pharmacologic doses of hydroxocobalamin (OH-Cbl) (1 mg) in the new or undefined patient. Dialysis may be necessary in some cases. Carnitine supplementation is useful, not only to reverse the deficiency of free carnitine that regularly occurs in these conditions, but also to form carnitine esters of accumulated toxic CoA esters; these carnitine esters are subsequently excreted in the urine.^{3,168}

Two treatment regimens for children with methylmalonic acidemia exist for chronic therapy and should be used in tandem. A diet restricted in protein (or a special formula restricted in precursors of methylmalonate) should be instituted as soon as life-threatening problems such as ketoacidosis, hypoglycemia, or hyperammonemia have been addressed;^{2,60} and supplementary Cbl (1-2 mg CN-Cbl or, preferably, OH-Cbl intramuscularly daily for several days) should be given as soon as the diagnosis of methylmalonic acidemia is seriously considered. Such measures should decrease the circulating concentrations of methylmalonate and propionate. Even Cbl-unresponsive children with delayed development have been shown to improve markedly when treated with careful dietary protein restriction.^{169,170} **Table 10** lists several other treatment options that may be successful.

TABLE 10. ADDITIONAL TREATMENTS IN CHRONIC THERAPY FOR METHYLMALONIC ACIDEMIA THAT MAY SUPPLEMENT CONVENTIONAL THERAPY (I.E., DIETARY RESTRICTION AND CBL SUPPLEMENTS).
L-carnitine supplementation ^{3,168}
Oral antibiotic therapy ¹⁷¹⁻¹⁷⁴
Total parenteral nutrition ¹⁷⁵
Allupurinol to treat uricacidemia ⁶⁰
Ascorbate to treat glutathione deficiency ⁸¹
Growth hormone to correct short stature and obesity ⁶⁰
Immunoglobulin therapy for brief tonic seizures ¹⁷⁶
Prenatal therapy with Cbl supplements (either to pregnant woman ^{163,177,178} or intrauterine ¹⁷⁹)

In addition, the successful treatment of *cblC*, *cblD*, or *cblF* patients may demand administration of very large amounts of Cbl.^{126,129,131-133,140,180} Such treatment has resulted in dramatic decreases in methylmalonate (less dramatic changes in urinary homocysteine) in patients who have received it.¹⁸¹ Additionally, supplementation with betaine, methionine, and carnitine can reduce homocysteine and organic acid levels, while alleviating many associated symptoms.^{180,182}

PROGNOSIS

The prognosis of patients with methylmalonic acidemia—either owing to isolated mutase deficiency (*mut*⁰, *mut*⁻, *cblA*, or *cblB*) or the forms with an additional methionine synthase deficiency (*cblC*, *cblD*, or *cblF*) depends on (a) early diagnosis and (b) the ability to effect good long-term metabolic control.¹ However some defects are less severe than others in terms of the outcome. Both the response to Cbl supplements and the long-term outcome in affected patients depend considerably on the nature of the biochemical lesion causing methylmalonic acidemia.⁴³ None of the children designated *mut*⁰ or *mut*⁻ responded to Cbl supplements with a distinct decrease in blood and urinary methylmalonate, whereas over 90% of the *cblA* and about 40% of the *cblB* patients showed a response. Moreover, one must alleviate the enzyme deficiency just slightly to see a positive response as studies suggest that raising enzyme activity to only 10 percent of normal via growth medium supplementation with OH-Cbl distinctly augments propionate pathway activity.^{95,159} The use of AdoCbl instead of CN-Cbl or OH-Cbl is ineffective.^{183,184}

Owing to complete methylmalonyl-CoA mutase deficiency the *mut*⁰ group has the poorest prognosis, with approximately 60% deceased and 40% distinctly impaired developmentally.^{43,44} In contrast, *cblA* patients (i.e., the group biochemically most responsive to

Cbl supplements) had the best outcome: 70% were alive and well at ages up to 14 years. The *cbIB* and *mut* groups were intermediate, with about equal fractions in each group being found in the alive and well, alive and impaired, or the deceased category.⁴³

Additionally, a long-term complication of methylmalonic acidemia patients is chronic renal failure.⁵⁰⁻⁵⁶ One report indicated that 8 of 12 non-Cbl responsive patients (1-9 years of age) had a reduced glomerular filtration rate, with five severely affected.⁵¹ In one of these, “greatly improved metabolic control” over a period of 18 months led to increased, but still impaired, renal function.⁵¹ It is not known what impact better metabolic control and Cbl supplementation may have in this and similar cases.¹

Finally, early diagnosis and prompt institution of therapy with Cbl supplements (and betaine) may be the only way to change the outcome of *cbIC*, *cbID*, and *cbIF* patients which has mostly been dismal thus far.^{126,127-143} At least one recent study reports on more favorable outcomes for eight *cbIC* patients subjected to aggressive therapy with intramuscular OH-Cbl, carnitine, and oral betaine.¹⁸²

CONCLUSION AND FUTURE DIRECTIONS

Methylmalonic acidemias are a group of rare but severe inborn errors of metabolism caused by non-functional methylmalonyl-CoA mutase. The resulting accumulation of organic and ketotic acid compounds precipitates a constellation of biochemical and pathologic problems which are often debilitating and fatal in infants and young children. Owing to the severity of the metabolic disorders of methylmalonic acidemia much work remains to be done towards the development of more effective treatments, and several paths toward doing so are being carved out.

Not surprisingly, liver transplantation has been attempted in a limited number of early-onset patients, who have the worst prognosis.¹⁸⁵⁻¹⁸⁸ Although liver transplantation appears to protect against acute metabolic decompensation, biochemical correction is incomplete and it is not certain that there will be complete protection against the renal and neurologic complications.¹ In one case, combined liver-kidney transplantation led to marked improvement, but the precise mechanism of its effectiveness remains to be worked out.¹⁸⁹

Preliminary steps have also been taken toward somatic gene therapy for mutase deficiency.^{190,191} Besides all the usual questions of safety and long-term stability of response that surround somatic gene therapy, two important issues remain unanswered for mutase: How much activity must be restored *in vivo* to normalize the biochemical hallmarks of the disease? And, does correction of the defect in the liver, for example, lead to reversal or amelioration of the pathologic changes in other organ systems and overall clinical improvement?

Another interesting concept involves the study of the molecular defects through an understanding of the enzyme structure.^{37,121,122} For example, it is known that certain mutations at the cobalamin binding site, sterically block the docking of the AdoCbl coenzyme, thus preventing holoenzyme formation.¹²² If one could chemically modify a Cbl derivative such that it could now fit into the binding site, then it would be possible to design specific pharmacologic agents against known structural defects of methylmalonyl-CoA mutase. In other words, such wonder drugs could restore compromised activity to methylmalonyl-CoA mutase in affected individuals to a significant degree, alleviating many symptoms of the disease. Approaches of this kind are important reasons for researchers to continue the molecular characterization of defects in methylmalonic acidemia.¹²¹

REFERENCES

1. Fenton, WA; Gravel, RA; Rosenblatt, DS: Disorders of Propionate and Methylmalonate Metabolism, in Charles R. Scriver, *et al.* (editors) *The metabolic and molecular basis of inherited disease*. Volume II, New York, McGraw-Hill, **2001**, p 2165.
2. Berry, GT: Inborn errors of amino acid and organic acid metabolism, in Cowett, RM (ed): *Principles of Perinatal and Neonatal Metabolism*, 2nd edition, New York, Springer **1998**, p 799.
3. Nyhan, W. L.; Haas, R. Inborn Errors of Amino Acid Metabolism and Transport, in Rosenberg, RN (ed): *Molecular and Genetic Basis of Neurological Disorders*, 2nd edition, Boston, Butterworth-Heinemann, **1997**, p 1129.
4. Smith, RM; Monty, KJ: Vitamin B₁₂ and propionate metabolism. *Biochem Biophys Res Commun* 1: 105, **1959**
5. Gurnani, S; Mistry, SP; Johnson, BC: Function of vitamin B₁₂ in methylmalonate metabolism. 1. Effect of a cofactor form of B₁₂ on the activity of methylmalonyl-CoA isomerase. *Biochim Biophys Acta* 38: 187, **1960**.
6. Stern, JR; Friedmann, DC: Vitamin B₁₂ and methylmalonyl-CoA isomerase. I. Vitamin B₁₂ and propionate metabolism. *Biochem Biophys Res Commun* 2:82, **1960**.
7. Levy, HL; Varvogli, L; Gabriela, MR; Waisbren, SE: High Cognitive Outcome in an Adolescent With Mut- Methylmalonic Acidemia. *Am J Med Genet* 96: 192, **2000**.
- 8.** Shapira, SK; Ledley, FD, Rosenblatt, DS; Levy, HL: Ketoacidotic crisis as a presentation of benign methylmalonic aciduria. *J Pediatr* 119: 80, **1991**.
9. Cox, EV; White, AM: Methylmalonic acid excretion: Index of vitamin B₁₂ deficiency. *Lancet* 2:853, **1962**.
10. Barness, LA; Young, D; Mellman, WJ; Kahn, SB; Williams, WJ: Methylmalonate excretion in patients with pernicious anemia. *New Engl J Med* 268: 144, **1963**.
11. Oberholzer, VC; Levin, B; Burgess, EA; Young, WF: Methylmalonic aciduria: An inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* 42: 492, **1967**.
12. Stokke, O; Eldjarn, L; Norum, KR, Steen-Johnsen, J; Halvorsen, S: Methylmalonic aciduria: A new inborn error of metabolism which may cause fatal acidosis in the neonatal period. *Scand J Clin Lab Invest* 20: 313, **1967**.
13. Childs, B; Nyhan, WL; Borden, M; Bard, L; Cooke, RE: Idiopathic hyperglycinuria: New disorder of amino acid metabolism I. *Pediatrics* 27: 522, **1961**.
14. Rosenberg, LE; Lilljeqvist, A-C; Hsia, YE: Methylmalonic aciduria: An inborn error leading to metabolic acidosis, long-chain ketonuria and intermittent hyperglycinemia. *New Engl J Med* 278: 1319, **1968**.
15. Hsia, YE; Scully, KJ; Rosenberg, LE: Defective propionate carboxylation in ketotic hyperglycinaemia. *Lancet* 1: 757, **1969**.
16. Lindblad, B; Olin, P; Svanberg, B, Zetterstrom, R: Methylmalonic acidemia. *Acta Paediatr Scand* 57: 417, **1968**.
- 17.** Lindblad, B; Lindstrand, K; Svanberg, B; Zetterstrom, R: The effect of cobamide coenzyme in the methylmalonic acidemia. *Acta Paediatr Scand* 58: 178, **1969**.
18. Minot, GR; Murphy, LP: Treatment of pernicious anemia by a special diet. *JAMA* 87: 470, **1926**.
19. Smith, EL: Purification of anti-pernicious anemia factors from liver. *Nature* 161: 638, **1948**.
20. Rickes, EL; Brink, NG; Koniuszy, FR; Wood, TR; Folkers, K: Crystalline vitamin B₁₂. *Science* 107: 396, **1948**.
21. Martson, HR; Allen, SH; Smith, RM: Primary metabolic defect supervening on vitamin B₁₂ deficiency in the sheep. *Nature* 190: 1085, **1961**.
22. Hodgkin, DC; Kamper, J; Mackay, M; Pickworth, J; Trueblood, KN; White, JG: Structure of vitamin B₁₂. *Nature* 178: 64, **1956**.
23. Rosenberg, LE; Lilljeqvist, AC; Hsia, YE; Rosenbloom, FM: Vitamin B₁₂ dependent methylmalonicaciduria: defective B₁₂ metabolism in cultured fibroblasts. *Biochem Biophys Res Commun* 37: 607, **1969**.
24. Mahoney, MJ; Rosenberg, LE; Mudd, SH; Uhlendorf, BW: Defective metabolism of vitamin B₁₂ in fibroblasts from children with methylmalonicaciduria. *Biochem Biophys Res Commun* 44: 375, **1971**.

25. Beck, WS; Flavin, M; Ochoa, S: Metabolism of propionic acid in animal tissues. III. Formation of succinate. *J Biol Chem* 229: 997, **1957**.
26. Kaziro, Y; Ochoa, S: The metabolism of propionic acid. *Adv Enzymol* 26: 283, **1964**.
27. Flavin, M, Ochoa, S: Metabolism of propionic acid in animal tissues. I. Enzymatic conversion of propionate to succinate. *J Biol Chem* 243: 1394, **1959**.
28. Sprecher, M; Clark, MJ; Sprinson, DB: The absolute configuration of methylmalonyl-CoA and stereochemistry of the methylmalonyl-CoA mutase reaction. *Biochem Biophys Res Commun* 15: 581, **1964**.
29. Retey, J; Lynen, F: The absolute configuration of methylmalonyl-CoA. *Biochem Biophys Res Commun* 16: 358, **1964**.
30. Tietz, A; Ochoa, S; Metabolism of propionic acid in animal tissues. V. Purification and properties of propionyl carboxylase. *J Biol Chem* 234: 1394, **1959**.
31. Mazumder, R; Sasakawa, T; Kaziro, Y; Ochoa, S: Metabolism of propionic acid in animal tissues. IX. Methylmalonyl coenzyme A racemase. *J Biol Chem* 237: 3065, **1962**.
32. Taylor, RT; Weissbach, H: Enzymatic synthesis of methionine: Formation of a radioactive cobamide enzyme with N⁵methyl-¹⁴C-tetrahydrofolate. *Arch Biochem Biophys* 119: 572, **1967**.
33. Mudd, SH, Levy, HL, Abeles, RH: A derangement in B₁₂ metabolism leading to homocystinemia, cystathioninemia and methylmalonicaciduria. *Biochem Biophys Res Commun* 35:121, **1969**.
34. Weissbach, H; Taylor, R: Role of vitamin B₁₂ in methionine biosynthesis. *Fed Proc* 25: 1649, **1966**.
35. Taylor, RT; Weissbach, H: Escherichia coli B N⁵-methyltetrahydrofolate-homocysteine vitamin-B₁₂ transmethylase: Formation and photolability of a methylcobalamin enzyme. *Arch Biochem Biophys* 123: 109, **1968**.
36. Behrman, N: *Textbook of Pediatrics*, 16th edition, W. B. Saunders Company, **2000**, p 361.
37. Mancia, F; Smith, GA; Evans, PR: Crystal Structure of Substrate Complexes of Methylmalonyl-CoA Mutase. *Biochemistry* 38: 7999, **1999**.
38. Ledley, FD; Lumetta, MR; Zoghbi, HY; VanTuinen, P; Ledbetter, SA; Ledbetter, DH: Mapping of Human Methylmalonyl-CoA Mutase (MUT) Locus on Chromosome 6. *Am J Hum Genet* 42: 839, **1988**.
39. Nham, S; Wilkmeyer, MF; Ledley, FD: Structure of the Human Methylmalonyl-CoA Mutase (MUT) Locus. *Genomics* 8: 710, **1990**.
40. Jansen, R; Ledley, FD: Heterozygous Mutations at the *mut* Locus in Fibroblasts with *mut*⁰ Methylmalonic Acidemia Identified by Polymerase-Chain-Reaction cDNA Cloning. *Am J Hum Genet* , 47: 808, **1990**.
41. Jansen, R; Kalousek, F; Fenton, WA; *et al.*: Cloning of full-length methylmalonyl-CoA mutase from a cDNA library using the polymerase chain reaction. *Genomics* 4: 198, **1989**.
42. Mancia, F; Keep, NH; Nakagawa, A; Leadley, PF; McSweeney, S; Rasmussen, B; Bosecke, P; Diat, O; Evans, PR: How coenzyme B₁₂ radicals are generated: The crystal structure of methylmalonyl-CoA mutase at 2 Å resolution. *Structure* 4: 339, **1996**.
43. Matsui, SM; Mahoney, MJ; Rosenberg, LE: The natural history of the inherited methylmalonic acidemias. *New Engl J Med* 308: 857, **1983**.
44. Shevell, MI; Matiaszuk, N; Ledley, FD; Rosenblatt, DS: Varying neurological phenotypes among *mut*⁰ and *mut*⁻ patients with methylmalonyl-CoA mutase deficiency. *Am J Med Genet* 45: 619, **1993**.
45. Rosenberg, LE: Disorders of propionate, methylmalonate, and cobalamin metabolism, in Stanbury, JB; Wyngarrden, JB; Friedrickson, DS (eds): *The metabolic basis of inherited disease*, 4th ed., New York, McGraw-Hill, **1978**, p 411.
46. Korf, B; Wallman, JK; Levy, HL: Bilateral lucency of the globus pallidus complicating methylmalonic acidemia. *Ann Neurol* 20: 364, **1986**.
47. Morrow, G III; Burkel, GM: Long-term management of a patient with vitamin B₁₂-responsive methylmalonic acidemia. *J Pediatr* 96: 425, **1980**.
48. Thompson, GN; Christodoulou, J; Danks, DM; Metabolic stroke in methylmalonic acidemia. *J Pediatr* 115: 499, **1989**.
49. Cowan, WJ; Wara, DW; Packman, S; *et al.*: Multiple biotin-dependent carboxylase deficiencies associated with defects in T-cell and B-cell immunity. *Lancet* 2: 115, **1979**.
50. Broyer, M; Guesry, P; Burgers, EA; *et al.*: Acidemic methyl malonique avec nephropathic hyperuricemique. *Arch Fr Pediatr* 31: 543, **1974**.
51. Walter, JH; Michalski, A; Wilson, WM; Leonard, JV; Barrat, TM; Dillon, MJ: Chronic renal failure in methylmalonic acidaemia. *Eur J Pediatr* 148: 344, **1989**.
52. Molteni, KH; Oberly, TD; Wolff, JA; Friedman, AL: Progressive renal insufficiency in methylmalonic acidemia. *Pediatr Nephrol* 5: 323, **1991**.

53. Whelan, DT; Ryan, E; Spate, M; *et al.*: Methylmalonic acidemia: 6 years' clinical experience with two variants unresponsive to vitamin B₁₂ therapy. *Can Med Assoc J* 120: 1230, **1979**.
54. Morita, J; It, Y; Yoshino, M, *et al.*: Persistent hyperkalemia in vitamin B₁₂ unresponsive methylmalonic acidemia. *J Inher Metab Dis* 12: 89, **1989**.
55. Wolff, JA; Strom, C; Griswold, W; *et al.* Renal tubular acidosis in methylmalonic acidemia. *J Neurogenet* 2: 31, **1991**.
56. Rutledge, SL; Geraghty, M; Mroczek, E; Rosenblatt, D; Kohout, E: Tubulointerstitial nephritis in methylmalonic aciduria. *Pediatr Nephrol* 7: 81, **1993**.
57. Trinh, B; Melhem, ER; Barker, PB: Multi-slice Proton MR Spectroscopy and Diffusion-weighted Imaging in Methylmalonic Acidemia: Report of Two Cases and Review of Literature. *AJNR Am J Neuroradiol* 22: 831, **2001**.
58. Wajner, M; Coelho, JC: Neurological dysfunction in methylmalonic acidemia is probably related to the inhibitory effect of methylmalonate on brain energy production. *J Inher Metab Dis* 20: 761, **1997**.
59. Scalabrino, G: Subacute combined degeneration one century later. The neurotrophic action of cobalamin (Vitamin B-12) revisited. *J Neuropath Exp Neuro* 60: 109, **2001**.
60. Goetz: *Textbook of Clinical Neurology*, 1st ed., W. B. Saunders Company, **1999**, p. 591.
61. Inoue, S; Krieger, I; Sarnaik, A; Ravindranath, Y; Fracassa, M; Ottenbreit, MJ: Inhibition of bone marrow stem cell growth *in vitro* by methylmalonic acid: A mechanism for pancytopenia in a patient with methylmalonic acidemia. *Pediatr Res* 15: 95, **1981**.
62. Yamaguchi, K.; Hirabayashi, K.; Honma, K. Methylmalonic acidemia—brain lesions in a case of vitamin B-12 non-responsive (*mut(0)*) type. *Clin Neuropath* 14: 216, **1995**.
63. Stromme, P; Stokke, O; Jellum, E; Skjeldal, OH; Baumgartner, R: Atypical methylmalonic aciduria with progressive encephalopathy, microcephaly and cataract in two siblings—a new recessive syndrome. *Clin Genet* 48: 1, **1995**.
64. Wyse, ATS; Streck, EL; Barros, SVT; Brusque, AM; Zugno, AI; Wajner, M: Methylmalonate administration decreases Na⁺,K⁺-ATPase activity in cerebral cortex of rats. *Neuroreport* 11: 2331, **2000**.
65. Demattosdutra, A; Defreitas, MS; Schroder, N; Zilles, AC; Wajner, M; Pessopatureur, R: Methylmalonic acid reduces the *in vitro* phosphorylation of cytoskeletal proteins in the cerebral cortex of rats. *Brain Res* 763: 221, **1997**.
66. Brusque, AM; Rotta, L; Pettenuzzo, LF; Junqueira, D; Schwarzbald, CV; Wyse, AT; Wannmacher, CMD; Dutra, CS; Wajner, M: Chronic postnatal administration of methylmalonic acid provokes a decrease of myelin content and ganglioside N-acetylneuraminic acid concentration in cerebrum of young rats. *Brazil J Med Biol Res* 34: 227, **2001**.
67. Msall, M; Batshaw, ML; Suss, R; Brusilow, SW; Mellits, ED: Neurologic Outcome in Children with Inborn Errors of Urea Synthesis: Outcome of Urea-Cycle Enzymopathies. *N Eng J Med* 310: 1500, **1984**.
68. Ledley, FD; Levy, HL; Shih, VE; Benjamin, R; Mahoney, MJ: Benign methylmalonic aciduria. *New Engl J Med* 311: 1015, **1984**.
69. Sniderman, LC; Lamber, M; Giguere, R; Auray-Blais, C; Lemieux, B; Laframboeise, R; Rosenblatt, DS; *et al.*: Outcome of individuals with low-moderate methylmalonic aciduria detected through a neonatal screening program. *J Pediatr* 134: 680, **1999**.
70. Feillet, F; Bodamer, OAF; Dixon, M.A; Sequeira, S; Leonard, JV: Resting energy expenditure in disorders of propionate metabolism. *J. Pediatr* 136: 659, **2000**.
71. Mayatepek, E; Hoffmann, GF; Baumgartner, R; Schulze, A; Jacobs, C; Trefz, FK; Bremer, HJ: Atypical vitamin B₁₂-unresponsive methylmalonic aciduria in a sibship with severe progressive encephalopathy: a new genetic disease? *Eur J Pediatr* 155: 398, **1996**.
72. Roe, CR; Struys, E; Kok, RM; Roes, DS; Jakobs, C: Methylmalonic semialdehyde dehydrogenase deficiency: Psychomotor delay and methylmalonic aciduria without metabolic decomposition. *J Inher Metab Dis* 21: 54, **1998**.
73. Ando, T; Rasmussen, K; Nyhan, WL; Wright, JM: Isolation and identification of methylcitrate, a major metabolic product of propionate in patients with propionic acidemia. *J Biol Chem* 247: 2200, **1972**.
74. Rasmussen, K; Ando, T; Nyhan, WL; Hull, D; Cotton, D; Wadlington, W; Kilroy, AW: Excretion of propionylglycine in propionic acidemia. *Clin Sci* 42: 665, **1972**.
75. Ando, T; Rasmussen, K; Nyhan, WL; Donnell, GN; Barnes, ND: Propionicacidemia in patients with ketotic hyperglycinemia. *J Pediatr* 78: 827, **1971**.
76. Stokke, O; Jellum, E; Eldjarn, L; Schnitler, R: The occurrence of hydroxy-*n*-valeric acid in a patient with propionic and methylmalonic acidemia. *Clin Chim Acta* 45: 391, **1973**.

77. Steinman, L; Clancy, RR; Cann, H; Urich, H: The Neuropathology of Propionic Acidemia. *Dev Med Child Neuro* 25: 87, **1983**.
78. Hsia, YE; Scully, K; Lilljeqvist, A-CH; Rosenberg, LE: Vitamin B₁₂ dependent methylmalonicaciduria. *Pediatrics* 46: 497, **1970**.
79. Utter, MF; Keech, DB; Scrutten, ML: A Possible role for acetyl CoA in the control of gluconeogenesis, in Webber G (ed): *Advances in Enzyme Regulation*, Vol. 2, New York, Pergamon, **1964**, p 49.
80. Halperin, ML, Schiller, CM; Friz, IB: The inhibition by methylmalonic acid of malate transport by the dicarboxylate carrier in rat liver mitochondria: A possible explanation for hypoglycemia in methylmalonic aciduria. *J Clin Invest* 50: 2276, **1971**.
81. Treacy, E; Arbour, L; Chessex, P; Graham, G; Kasprzak, L; Casey, K; Bell, L; Mamer, O; Scriver, CR: Glutathione deficiency as a complication of methylmalonic acidemia—response to high doses of ascorbate. *J Pediatr* 129: 445, **1996**.
82. Hillman, RE; Sowers, LH; Cohen JL: Inhibition of glycine oxidation in cultured fibroblasts by isoleucine. *Pediatr Res* 7: 945, **1973**.
83. Hillman, RE; Otto, EF: Inhibition of glycine-serine interconversion in cultured human fibroblasts by products of isoleucine catabolism. *Pediatr Res* 9: 941, **1974**.
84. Glasgow, AM; Chase, HP: Effect of propionic acid on fatty acid oxidation and ureagenesis. *Pediatr Res* 10: 683, **1976**.
85. Stewart, PM; Walser, M: Failure of the normal ureagenic response to amino acids in organic acid loaded rats: a proposed mechanism for the hyperammonemia of propionic and methylmalonic acidemia. *J Clin Invest* 66: 484, **1980**.
86. Coude, FX; Sweetman, L; Nyhan, WL: Inhibition by propionyl CoA of N-acetylglutamate synthase in rat liver mitochondria. *J Clin Invest* 64: 1544, **1979**.
87. Kirkman, HN; Kiesel, JL: Congenital hyperammonemia. *Pediatr Res* 3: 358, **1969**.
88. Harris, DJ; Yang, BJ-Y; Snodgrass, PJ: Carbamyl phosphate synthetase deficiency: A possible transient phenocopy of dysautonomia. *Am J Hum Genet* 29: 52, **1977**.
89. Chalmers, RA; Roe, CR; Stacey, TE; *et al.*: Urinary excretion of l-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of l-carnitine. *Pediatr Res* 18: 1325, **1984**.
90. Libert, R; Van Hoof, F; Thillaye, M; Vincent, MF; Nassogne, MC; de Hoffmann, E; Schanck, A: Identification of undescribed medium-chain acylcarnitines present in urine of patients with propionic and methylmalonic acidemias. *Clinica Chimica Acta* 295: 87, **2000**.
91. Raff, ML; Crane, AM; Jansen, R; Ledley, FD; Rosenblatt, DS: Genetic Characterization of a MUT Locus Mutation Discriminating Heterogeneity in *mut*⁰ and *mut* Methylmalonic Aciduria by Interallelic Complementation. *J Clin Invest* 87: 203, **1991**.
92. Janata, J; Kogekar, N; Fenton, WA: Expression and kinetic characterization of methylmalonyl-CoA mutase from patients with the *mut*⁻ phenotype—evidence for naturally occurring interallelic complementation. *Hum Mol Genet* 6: 1457, **1997**.
93. Gravel, RA; Mahoney, MJ; Ruddle, FH; Rosenberg, LE: Genetic complementation in heterokaryons of human fibroblasts defective in cobalamin metabolism. *Proc Natl Acad Sci USA* 72: 3181, **1975**.
94. Willard, HF; Mellman, IS; Rosenberg, LE: Genetic complementation among inherited deficiency of the methylmalonyl-CoA mutase activity: Evidence for a new class of human cobalamin mutants. *Am J Hum Genet* 30: 1, **1978**.
95. Willard, HF; Rosenberg, LE: Inborn errors of cobalamin metabolism: Effect of cobalamin supplementation in culture on methylalonyl-CoA mutase activity in normal and mutant human fibroblasts. *Biochem Genet* 17: 57, **1979**.
96. Willard, HF; Rosenberg, LE: Inherited methylmalonyl-CoA mutase apoenzyme deficiency in human fibroblasts: Evidence for allelic heterogeneity, genetic compounds, and codominant expression. *J Clin Invest* 65: 690, **1980**.
97. Fenton, WA, Rosenberg, LE: The defect in cblB class of human methylmalonic acidemia: Deficiency of cob(I)alamin adenosyltransferase activity in extracts of cultured fibroblasts. *Biochem Biophys Res Commun* 98: 283, **1981**.
98. Ledley, FD; Crane, AM; Lumetta, M: Heterogeneous alleles and expression of methylmalonyl-CoA mutase in *mut* methylmalonic acidemia. *Am J Hum Genet* 6: 539, **1990**.
99. Coulombe, JT; Shih, VE; Levy, HL: Massachusetts Metabolic Disorders Screening Program. II. Methylmalonic aciduria. *Pediatrics* 67: 26, **1981**.

100. MacMahon, M: Requesting vitamin B₁₂ and folate assays. *Lancet* 346: 973, **1995**.
101. Morrow, G 3rd; Barness, LA; Cardinale, GJ; Abeles, RH; Flaks, JG: Congenital methylmalonic acidemia: Enzymatic evidence for two forms of disease. *Proc Natl Acad Sci USA* 63: 191, **1969**.
102. Morrow, G 3rd; Mahoney, MJ; Mathews, C; Lebowitz, J: Studies of methylmalonyl coenzyme A carbonylmotase activity in methylmalonic acidemia. I. Correlation of clinical, hepatic, and fibroblast data. *Pediatr Res* 9: 641, **1975**.
103. Kolhouse, JF; Utley, C; Fenton, WA; Rosenberg, LE: Immunochemical studies on cultured fibroblasts from patients with inherited methylmalonic acidemia. *Proc Natl Acad Sci USA* 78: 7737, **1981**.
104. Fenton, WA; Hack, AM; Kraus, JP; Rosenberg, LE: Immunochemical studies of fibroblasts from patients with methylmalonyl-CoA mutase apoenzyme deficiency: Detection of a mutation interfering with mitochondrial import. *Proc Natl Acad Sci USA* 84: 1421, **1987**.
105. Ledley, FD; Jansen, R; Nham, SU; Fenton, WA; Rosenberg, LE: Mutation eliminating mitochondrial leader sequence of methylmalonyl CoA mutase causes mut⁰ methylmalonic aciduria. *Proc Natl Acad Sci USA* 87: 3147, **1990**.
106. Willard, HF; Rosenberg, LE: Inherited deficiencies of human methylmalonyl-CoA mutase activity: Reduced affinity of mutant apoenzyme for adenosylcobalamin. *Biochem Biophys Res Commun* 74: 927, **1977**.
107. Morrow, G III; Revsin, B; Clark, R; Lebowitz, J; Whelen, DT: A new variant of methylmalonic acidemia: Defective coenzyme-apoenzyme binding in cultured fibroblasts. *Clin Chem Acta* 85: 67, **1978**.
108. Thoma, NH; Leadlaey, PF: Homology modeling of human methylmalonyl-CoA mutase: A structural basis for point mutations causing methylmalonic aciduria. *Protein Sci* 5: 1922, **1996**.
109. Qureshi, AA; Crane, AM; Matiaszuk, NV; Resvani, I; Ledley, FD; Rosenblatt, DS: Cloning and expression of mutations demonstrating intragenic complementation in mut⁰ methylmalonic aciduria. *J Clin Invest* 93: 1812, **1994**.
110. Crane, AM; Ledley, FD: Cluster of mutations within methylmalonyl CoA mutase associated with *mut^f* methylmalonic acidemia, intra-allelic complementation, and impaired adenosylcobalamin binding. *Am J Hum Genet* 55: 42, **1994**.
111. Crane, A; Martin, LS; Valle, D; Ledley, FD: Phenotype of disease in three patients with identical mutations in methylmalonyl CoA mutase. *Hum Genet* 89: 259, **1992**.
112. Ogasawara, M; Matsubara, Y; Mikami, H; Narisawa, K: Molecular analysis of methylmalonic aciduria: Identification of novel mutations in the methylmalonyl CoA mutase gene with decreased level of mutant mRNA. *Am J Hum Genet* 55: A233, **1994**.
113. Ogasawara, M; Matsubara, Y; Mikami, H; Narisawa, KK: Identification of two novel mutations in the methylmalonyl-CoA mutase gene with decreased levels of mutant mRNA in methylmalonic acidemia. *Hum Mol Genet* 3: 867, **1994**.
114. Touranine, RL; Rolland, MO; Divry, P; Mathieu, M; Guilbaud, P; Botton, D: A 13 bp deletion (1952del13) in the methylmalonyl CoA mutase gene of an affected patient. *Hum Mutat* 5: 354, **1995**.
115. Crane, AM; Jansen, R; Andrews, ER; Ledley, FD: Cloning and expression of a mutant methylmalonyl coenzyme A mutase with altered cobalamin affinity that causes *mut^f* methylmalonic aciduria. *J Clin Invest* 89: 385, **1992**.
116. Adjalla, CE; Hosack, AR; Matiaszuk, NV; Rosenblatt, DS: A common mutation among blacks with *mut^f* methylmalonic aciduria. *Hum Mut Suppl* 1: 248, **1998**.
117. Acquaviva, C; Benoist, JF; Callebaut, I; Guffon, N; de Baulny, HO; Touati, G; Aydin, A; Porquet, D; Elion, J: N219Y, a new frequent mutation among *mut* degrees forms of methylmalonic acidemia in Caucasian patients. *Eur J Hum Genet* 9: 577, **2001**.
118. Adjalla, CE; Hosack, AR; Gilfix, BM; Sun, S; Chan, A; Evans, S; Matiaszuk, NV; *et al.*; Seven novel mutations in *mut* methylmalonic aciduria. *Hum Mut* 11: 270, **1998**.
119. Benoist, JF; Acquaviva, C; Callebaut, I; Guffon, N; de Baulny, HO; Mornon, JP; Porquet, D; Elion, J: Molecular and structural analysis of two novel mutations in a patient with Mut(-) methylmalonyl-CoA mutase deficiency. *Mol Genet Metab* 72: 181, **2001**.
120. Mikami, H.; Ogasawara, M.; Matsubara, Y.; Kikuchi, M.; Miyabayashi, S.; Kure, S.; Narisawa, K. Molecular analysis of methylmalonyl-CoA mutase deficiency: identification of three missense mutations in *mut(0)* patients. *J Hum Genet* 44: 35, **1999**.
121. Ledley, FD; Rosenblatt, DS: Mutations in *mut* Methylmalonic Acidemia: Clinical and Enzymatic Correlations. *Hum Mut* 9: 1, **1997**.

122. Drennan, CL; Matthews, RG; Rosenblatt, DS; Ledley, FD; Fenton, WA; Ludwig, ML: Molecular basis for dysfunction of some mutant forms of methylmalonyl-CoA mutase: Deductions from the structure of methionine synthase. *Proc Natl Acad Sci USA* 93: 5550, **1996**.
123. Mahoney, MJ; Hart, AC; Steen, VD; Rosenberg, LE: Methylmalonic acidemia: Biochemical heterogeneity in defects of 5'-deoxyadenosyl-cobalamin synthesis. *Proc Natl Acad Sci USA* 72: 2799, **1975**.
124. Watanabe, F; Saido, H; Yamaji, R; Miyatake, K; Isegawa, Y; Ito, A; Yubisui, T; *et al.*: Mitochondrial NADH- or NADP-linked aquacobalamin reductase activity is low in human skin fibroblasts with defects in synthesis of cobalamin coenzymes. *J Nutr* 126: 2947, **1996**.
125. Cooper, BA; Rosenblatt, DS; Watkins, D: Methylmalonic aciduria due to a new defect in adenosylcobalamin accumulation by cells. *Am J Hematol* 34: 115, **1990**.
126. Goodman, SI; Moe, PG; Hammond, KB; Mudd, SH; Uhlendorff, BW: Homocystinuria with methylmalonic aciduria: Two cases in a sibship. *Biochem Med* 4: 500, **1970**.
127. Levy, HL; Mudd, SH; Schulman, JD; Dreyfus, PM; Abeles, RH: A derangement in B₁₂ metabolism associated with homocystinemia, cystathioninemia, hypomethioninemia and methylmalonic aciduria. *Am J Med* 48: 390, **1970**.
128. Dillon, MJ; England, JM; Gompertz, D; Goodey, PA; Grant, DB; Hussein, HA; Linnell, JC; *et al.*: Mental retardation, megaloblastic anemia, methylmalonic aciduria and abnormal homocysteine metabolism due to an error in vitamin B₁₂ metabolism. *Clin Sci Mol Med* 47: 43, **1974**.
129. Anthony, M; Mcleay, AC: A unique case of derangement of vitamin B₁₂ metabolism. *Proc Aust Assoc Neurol* 13: 61, **1976**.
130. Baumgartner, ER; Wick, H; Maurer, R; Egli, N; Steinmann, B: Congenital defect in intracellular cobalamin metabolism resulting in homocystinuria and methylmalonic aciduria. I. Case report and histopathology. *Helv Paediatr Acta* 34: 465, **1979**.
131. Carmel, R; Bedros, AA; Mace, JW; Goodman, SI: Congenital methylmalonic aciduria-homocystinuria with megaloblastic anemia: Observations on response to hydroxocobalamin and on the effect of homocysteine and methionine on the deoxyuridine suppression test. *Blood* 55: 570, **1980**.
132. Shinnar, S; Singer, HS: Cobalamin C mutation (methylmalonic aciduria and homocystinuria) in adolescence: A treatable cause of dementia and myelopathy. *New Engl J Med* 311: 451, **1984**.
133. Mitchell, GA; Watkins, D; Melancon, SB; Rosenblatt, DS; Geoffroy, B; Orquin, J; Homsy, MB; *et al.*: Clinical heterogeneity in cobalamin C variant of combined homocystinuria and methylmalonic aciduria. *J Pediatr* 108: 410, **1986**.
134. Cogan, DG; Schulman, J; Porter, RJ; Mudd, SH: Epileptiform ocular movements with methylmalonic aciduria and homocystinuria. *Am J Ophthalmol* 90: 251, **1980**.
135. Linnell, JC; Miranda, B; Bhatt, HR; Downton, SB; Levy, HL: Abnormal cobalamin metabolism in a megaloblastic child with homocystinuria, cystathioninuria, and methylmalonic aciduria. *J Inherit Metab Dis* 6(suppl 2): 137, **1983**.
136. Mamlock, RJ; Isenberg, JN; Rassin, DN: A cobalamin metabolism defect with homocystinuria, methylmalonic aciduria and macrocytic anemia. *Neuropediatrics* 17: 94, **1986**.
137. Ravindranath, Y; Krieger, I: Vitamin B₁₂ (Cbl) and folate interrelationship in a case of homocystinuria-methylmalonic (HC-MMA)-uria due to genetic deficiency. *Pediatr Res* 18: 247a, **1984**.
138. Ribes, A; Vilaseca, A; Briones, P; Maya, A; Sabater, J; Pascual, P; Alvarez, L; Ros, J; Gonzalez, Pascual E: Methylmalonic aciduria with homocystinuria. *J Inherit Metab Dis* 7: 129, **1984**.
139. Robb, RM; Downton, SB; Fulton AB; Levy, HL: Retinal degeneration in vitamin B₁₂ disorder associated with methylmalonic aciduria and sulfur amino acid abnormalities. *Am J Ophthalmol* 97: 691, **1984**.
140. Rosenblatt, DS; Laframboise, R; Pichette, J; Langevin, P; Cooper, BA; Costa, T: New disorder of vitamin B₁₂ metabolism (cobalamin F) presenting as methylmalonic aciduria. *Pediatrics* 78: 51, **1986**.
141. Shih, VE; Axel, SM; Tewksbury, JC; Watkins, D; Cooper, BA; Rosenblatt, DS: Defective lysosomal release of vitamin B₁₂ (cblF): A hereditary metabolic disorder associated with sudden death. *Am J Med Genet* 33: 555, **1989**.
142. Wong, LTK; Rosenblatt, DS; Applegarth, DA, Davidson, AGF: Diagnosis and treatment of a child with cblF disease. *Clin Invest Med* 15(suppl): 111, **1992**.
143. MacDonald, MR; Wiltse, HE; Bever, JL; Rosenblatt, DS: Clinical heterogeneity in two patients with cblF disease. *Clin Invest Med* 51: 353, **1992**.
144. Watkins, D; Rosenblatt, DS: Failure of lysosomal release of vitamin B₁₂: a new complementation group causing methylmalonic aciduria (cblF). *Am J Hum Genet* 39: 404, **1986**.

145. Rosenblatt, DS; Aspler, AL; Shevell, MI; Pletcher, BA; Fenton, WA, Seashore, MR: Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). *J Inherit Met Dis* 20: 528, **1997**.
146. Howard, R; Frieden, IJ; Crawford, D; Mccalmon, T; Levy, ML; Rosenblatt, DS; Sweetman, L; Goodman, SI; Ohnstad, C; Hart, K; Berrios, M; Packman, S: Methylmalonic acidemia, cobalamin C type, presenting with cutaneous manifestations. *Arch Dermatol* 133: 1563, **1997**.
147. Waggoner, DJ; Ueda, K; Dowton, SB: Methylmalonic Aciduria (cblF): Case Report and Response to Therapy. *Am J Med Genet* 79: 373, **1998**.
148. Linnell, JC; Matthews, DM; Mudd, SH; Ulendorf, BW; Wise, IJ; Cobalamins in fibroblasts cultured from normal control subjects and patients with methylmalonic aciduria. *Pediatr Res* 10: 179, **1976**.
149. Baumgartner, ER; Wick, H; Linnell, JC; Gaull, GE; Bachmann, C; Steinmann, B: Congenital defect in intracellular cobalamin metabolism resulting in homocystinuria and methylmalonic aciduria. II. Biochemical investigations. *Helv Paediatr Acta* 34: 483, **1979**.
150. Rosenberg, LE; Patel, L; Lilljeqvist, A: Absence of an intracellular cobalamin binding protein in cultured fibroblasts from patients with defective synthesis of 5' deoxyadenosylcobalamin and methylcobalamin. *Proc Natl Acad Sci USA* 72: 4617, **1975**.
151. Mellman, IS; Lin, P-F; Ruddle, FH; Rosenberg, LE: Genetic control of cobalamin binding in normal and mutant cells: Assignment of the gene for 5-methyltetrahydrofolate: L-homocysteine S-methyltransferase to human chromosome 1. *Proc Natl Acad Sci USA* 76: 405, **1979**.
152. Mudd, SH; Uhlenendorf, BW; Hinds, KR; Levy, HL: Deranged B₁₂ metabolism: Studies of fibroblasts grown in tissue culture. *Biochem Med* 4: 215, **1970**.
153. Andersson, HC; Shapira, E: Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cblC). *J Pediatr* 132: 121, **1998**.
154. Mellman, IH; Willard, HF; Youngdahl-Turner, P; Rosenberg, LE: Cobalamin coenzyme synthesis in normal and mutant human fibroblasts: Evidence for a processing enzyme activity deficient in cblC cells. *J Biol Chem* 254: 11847, **1979**.
155. Pezacka, EH: Identification and characterization of two enzymes involved in the intracellular metabolism of cobalamin: Cyanocobalamin beta-ligand transferase and microsomal cob(III)alamin reductase. *Biochim Biophys Acta* 1157: 167, **1993**.
156. Pezacka, EH; Rosenblatt, DS: Intracellular metabolism of cobalamin. Altered activities of β -axial-ligand transferase and microsomal cob(III)alamin reductase in cblC and cblD fibroblasts, in Bhatt, HR; James, VHT; Besser, GM; Bottazzo, GF; Keen, H (eds): *Advances in Thomas Addison's Diseases*. London, Bristol, Journal of Endocrinology, **1994**, p 315.
157. Pezacka, E; Green, R; Jacobsen, DW: Glutathionylcobalamin as an intermediate in the formation of cobalamin coenzymes. *Biochem Biophys Res Commun* 169: 443, **1990**.
158. Powers, JM; Rosenblatt, DS; Schmidt, RE; Cross, AH; Black, JT; Moser, AB; Moser, HW Morgan, DJ: Neurological and neuropathologic heterogeneity in two brothers with cobalamin C deficiency. *Annals Neuro* 49: 396, **2001**.
159. Willard, HF; Rosenberg, LE: Inherited deficiencies of human methyl-malonyl CoA mutase: Biochemical and genetic studies in cultured skin fibroblasts, in Hommes, FA (ed): *Models for the Study of Inborn Errors of Metabolism*. Amsterdam, Elsevier North-Holland, **1979**, p 297.
160. Rosenblatt, DS; Cooper, BA: Inherited disorders of vitamin B₁₂ metabolism. *Blood Rev* 1: 177, **1987**.
161. Naylor, EW; Chace, DH: Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neuro* 14: S4, **1999**.
162. Morrow, G; Schwartz, RH; Hallock, JA; Barness, LA: Prenatal detection of methylmalonic acidemia. *J Pediatr* 77: 120, **1970**.
163. Ampola, MG; Mahoney, MJ; Nakamura, E; Tanaka, K: Prenatal therapy of a patient with vitamin B₁₂ responsive methylmalonic acidemia. *New Engl J Med* 293: 313, **1975**.
164. Mahoney, MJ; Rosenberg, LE; Linblad, B; Waldenstrom, J; Zetterstrom, R: Prenatal diagnosis of methylmalonic aciduria. *Acta Paediatr Scand* 64: 44, **1975**.
165. Willard, HF; Ambani, LM; Hart, AC; Mahoney, MJ; Rosenberg, LE: Rapid prenatal and postnatal detection of inborn errors of propionate, methylmalonate, and cobalamin metabolism: A sensitive assay using cultured cells. *Hum Genet* 34: 277, **1976**.
166. Morrow, G; Revsin, B; Mathews, C; Giles H: A simple rapid method for prenatal detection of defects in propionate metabolism. *Clin Genet* 10: 218, **1976**.

167. Hack, AM; Fenton, WA: unpublished results.
168. Roe, CR; Hoppel, CL; Stacey, TE; et al.: Metabolic response to carnitine in methylmalonic aciduria. An effective strategy for elimination of propionyl groups. *Arch Dis Child* 58: 916, **1983**.
169. Nyhan, WL; Fawcett, N; Ando, T; Rennert, OM; Julius, RL: Response to dietary therapy in B₁₂ unresponsive methylmalonic acidemia. *Pediatrics* 51: 539, **1973**.
170. Satoh, T; Narisawa, K; Igarashi, Y; Saitoh, T; Hayasaka, K; Ichinohazama, Y; Onodera, H; Tada, K; Oohara, K: Dietary therapy in two patients with vitamin B₁₂-unresponsive methylmalonic acidemia. *Eur J Pediatr* 135: 305, **1981**.
171. Leonard, JV: Stable Isotope studies in propionic and methylmalonic acidemia. *Eur J Pediatr* 156 (Suppl 1): S67, **1997**.
172. Thompson, GN; Chalmers, RA; Walter, JH; Bresson, JL; Lyonnet, SL; Reed, PJ; Saudubray, JM; et al.: The use of metronidazole in management of methylmalonic and propionic acidemias. *Eur J Pediatr* 149: 792, **1990**.
173. Bain, MD; Jones, M; Borriello, SP; Reed, PJ; Tracey, BM; Chalmers, RA; Stacey, TE: Contribution of gut bacterial metabolism to human metabolic disease. *Lancet* 1: 1078, **1988**.
174. Koletzko, B; Bachmann, C; Wendel, U: Antibiotic therapy for improvement of metabolic control in methylmalonic aciduria. *J Pediatr* 117: 99, **1990**.
175. Kahler, SG; Millington, DS; Cederbaum, SD; Vargas, J; Bond, LD; Maltby, DA; Gale, DS; Roe, CR: Parenteral nutrition in propionic and methylmalonic acidemia. *J Pediatr* 15: 235, **1989**.
176. Aikoh, H; Sasaki, M; Sugai, K; Yoshida, H; Sakuragawa N: Effective immunoglobulin therapy for brief tonic seizures in methylmalonic acidemia. *Brain Dev* 19: 502, **1997**.
177. Van der Meer, SB; Spaapen, LJM; Fowler, B; Jakobs, C; Kleijer, WJ; Wendel, U: Prenatal treatment of a patient with vitamin B₁₂-responsive methylmalonic acidemia. *J Pediatr* 117: 923, **1990**.
178. Evans, MI; Duquette, DA; Rinaldo, P; Bawle, E; Rosenblatt, DS; Whity, J; Quintera, RA; Johnson, MP: Modulation of B₁₂ dosage and response in fetal treatment of methylmalonic aciduria (MMA): Titration of treatment dose to serum and urine MMA. *Fetal Diagn Ther* 12: 21, **1997**.
179. Geimbruch, U; Geipel, A: Direct and transplacental intrauterine fetal therapy. *Gynakologe* 32: 840, **1999**.
180. Bartholomew, DW; Batshaw, ML, Allen, RH; Roe, CR; Rosenblatt, D; Valle, DL; Franconmano, CA: Therapeutic approaches to cobalamin-C methylmalonic acidemia and homocystinuria. *J Pediatr* 112: 32, **1988**.
181. Bellini, C; Cerone, R; Bonacci, W; Caruso, C; Magliano, CP; Serra, G; Fowler, B; et al.: Biochemical diagnosis and outcome of 2 years treatment in a patient with combined methylmalonic aciduria and homocystinuria. *Eur J Pediatr* 151: 818, **1992**.
182. Andersson, HC; Marble, M; Shapira, E: Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. *Genet in Med* 1: 146, **1999**.
183. Batshaw, ML; Thomas, GH; Cohen, SR; Matalon, R; Mahoney, MJ: Treatment of the cbl B form of methylmalonic acidemia with adenocobalamin. *J Inher Metab Dis* 7: 65, **1984**.
184. Chalmers, RA; Bain, MD; Mistry, J; Tracey, BM; Weaver, C: Enzymologic studies on patients with methylmalonic aciduria: Basis for a clinical trial of deoxyadenosylcobalamin in a hydroxocobalamin unresponsive patient. *Pediatr Res* 30: 560, **1991**.
185. Leonard, JV: The management and outcome of propionic and methylmalonic acidemia. *J Inher Metab Dis* 18: 430, **1995**.
186. Nicolaides, P; Leonard, J; Surtees, R: Neurological outcome of methylmalonic acidemia. *Arch Dis Child* 78: 508, **1998**.
187. Wilcken, B; Carpenter, K; Dorney, S; Shun, A: Liver transplantation in methylmalonic aciduria. *J Inher Metab Dis* 21: 42, **1998**.
188. McKiernan, PJ; Preece, MA; Leonard, JV; Mayer, AD; Buckels, JAC: Liver transplantation in infancy for severe methylmalonic acidemia. *J Inher Metab Dis* 21: 42, **1998**.
189. Vanthoff, WG; Dixon, M; Taylor, J; Mistry, P; Rolles, K; Rees, L; Leonard, JV: Combined liver-kidney transplantation in methylmalonic acidemia. *J Pediatr* 132: 1043, **1998**.
190. Adams, RM; Soriano, HE; Wang, M; Darlington, G; Steffen, D; Ledley, FD: Transduction of primary human hepatocytes with amphotropic and xenotropic retroviral vectors. *Proc Natl Acad Sci USA* 89: 8981, **1992**.
191. Stankovics, J; Andrews, E; Wu, G; Ledley, FD: Overexpression of human methylmalonyl CoA mutase (MCM) in mouse liver after *in vivo* gene delivery using asialoglycoprotein complexes. *Am J Hum Genet* 51: 177, **1992**.