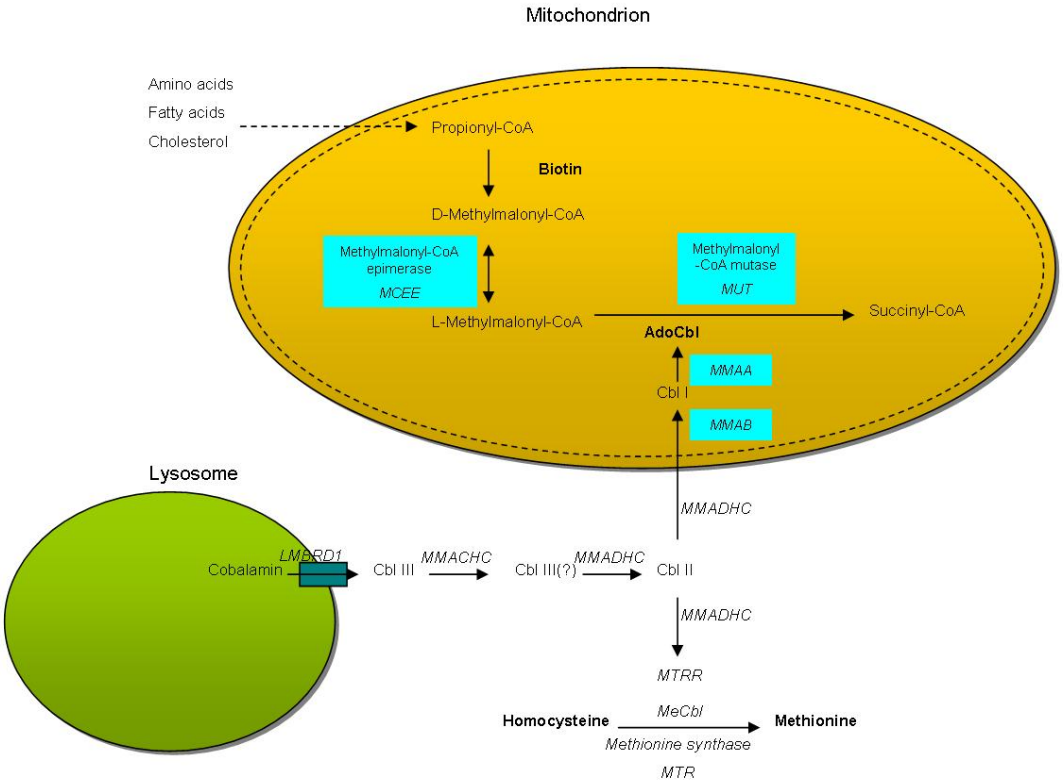
Genetic Testing for Methylmalonic Acidemia, Disorders of Intracellular Cobalamin Metabolism and Related Disorders

Panel Gene List: *ABCD4, ACSF3, AMN, CD320, CUBN, HCFC1, LMBRD1, MCEE, MLYCD, MMAA, MMAB, MMACHC, MMADHC, MTR, MTRR, MUT, SUCLA2, SUCLG1, TCN2*

Overview of Metabolic Pathway:

Propionyl-CoA is an end-product of several metabolic pathways: the breakdown of odd-chain fatty acids, amino acids and cholesterol. The major pathway to convert propionyl-CoA into the TCA-cycle intermediate, succinyl-CoA in the mitochondrial matrix requires the proper functioning of multiple enzymes. Propionyl-CoA carboxylase converts propionyl-CoA into *D*-methylmalonyl-CoA, which is then converted to *L*-methylmalonyl-CoA by methylmalonyl-CoA epimerase, encoded by the *MCEE* gene. *L*-methylmalonyl-CoA is converted to the TCA cycle intermediate succinyl-CoA by methylmalonyl-CoA mutase (MUT), which is encoded by the *MUT* gene. The MUT enzyme requires adenosyl-cobalamin (AdoCbl), an activated form of vitamin B_{12}, as a cofactor. Through a complex pathway involving absorption, transport systems and intracellular metabolism, vitamin B_{12} (cobalamin or cbl) is processed to adenosyl-cobalamin (AdoCbl), the cofactor for MUT, and methylcobalamin (MeCbl), the cofactor for methionine synthase (MTR). MTR is encoded by the *MTR* gene and catalyzes the methylation of homocysteine to generate methionine. Therefore, the conversion of cobalamin to AdoCbl and MeCbl is necessary for homeostasis of methylmalonic acid and homocysteine. The lysosomal membrane protein LMBD1, encoded by the *LMBRD1* gene, facilitates the export of cobalamin from the lysosomal membrane into the cytoplasm. Enzymes encoded by the *MMACHC*, *MMADHC*, *MMAB*, *MMAA* and *MTRR* genes are involved in the conversion of cobalamin to AdoCbl in the mitochondria and to MeCbl in the cytoplasm.



Adapted from Manoli, I. and Venditti, C. (2010)

Clinical Features:

Isolated Methylmalonic Acidemia:

Deficiency of the enzymes encoded by the *MUT*, *MMAA*, *MMAB*, *MMADHC*, *MCEE and TCN2* genes may result in isolated methylmalonic acidemia/aciduria. The clinical features of these disorders are highly variable with onset ranging from the neonatal period to adulthood.[1] Affected individuals have intermittent metabolic decompensation that is usually associated with intercurrennt infections and stress. The neonatal presentation may consist of lethargy, vomiting, hypotonia, hypothermia, respiratory distress, severe ketoacidosis, hyperammonemia, neutropenia, thrombocytopenia and can result in death.[1] The infantile/non-B_{12}-responsive phenotype is the most common form with infants appearing normal at birth and later presenting with lethargy, vomiting, dehydration, hepatomegaly, hypotonia and encephalopathy, while the less common intermediate B_{12}-responsive phenotype usually presents in the first months or years of life with anorexia, failure to thrive, hypotonia, developmental delay and protein aversion.[1] Other complications of methylmalonic acidemia may include developmental delay, renal failure, “metabolic stroke”, movement disorder, dystonia, pancreatitis, growth failure and optic nerve atrophy.[1]

Hyperhomocysteinemia/Homocystinuria:
Deficiency of the enzymes encoded by the *MMACHC*, *MMADHC*, *MTRR*, *LMBRD*, *MTR, ABCD4,* and *HCFC1* genes result in a highly variable group of disorders that may be characterized by hyperhomocysteinemia/homocystinuria. Defects in *MMACHC*, *MMADHC* and *LMBRD1* may also be associated with methylmalonic acidemia. Most of these disorders are quite rare; therefore, the clinical features are not well defined but may include megaloblastic anemia, failure to thrive, microcephaly, cerebral atrophy, lethargy, feeding difficulties, hypotonia, seizures, developmental delay, ataxia and optic atrophy. A Marfanoid appearance has been described in patients with variants in *MMADHC* while facial abnormalities and congenital heart defects have been reported in patients with variants in *LMBRD1*.[3, 4]

Related Disorders:
Variants in other genes that may also result in elevated methylmalonic acid include the *ACSF3, CD320*, *MLYCD*, *SUCLG1* and *SUCLA2* genes. The *ACSF3* gene encodes a methylmalonyl-CoA and malonyl-CoA synthetase.[12] Variants in the *ACSF3* gene have been identified in patients with combined malonic and methylmalonic aciduria (CMAMMA) which is characterized by elevations of urinary malonic acid that are higher than methylmalonic acid with normal malonyl-CoA decarboxylase enzyme activity.[11, 12] The symptoms of CMAMMA are variable. Some individuals exhibit no clinical symptoms while others have been reported with immunodeficiency, failure to thrive, neurological problems, microcephaly and developmental delay.[11, 12] The *CD320* gene encodes the transcobalamin receptor (TCBIR), that is expressed on the plasma membrane and binds cobalamin-saturated transcobalamin and transports it into the cells.[5] Very few individuals with TCBIR deficiency have been described, all of whom were asymptomatic newborns that were referred for analysis due to elevated C3-acylcarnitine levels identified on MS/MS newborn screening and subsequent identification of elevated methylmalonic acid in urine.[5] No clinical phenotype has been clearly associated with this disorder to date, and the long term consequences of this condition are not currently known.[5] The *MYLCD* gene encodes the malonyl-CoA decarboxylase enzyme, which catalyzes the decarboxylation of malonyl-CoA to acetyl-CoA. Deficiency of malonyl-CoA decarboxylase results in accumulation of malonyl-CoA, an inhibitor of numerous metabolic pathways. Symptoms include developmental delay, hypertrophic cardiomyopathy, seizures, acidosis, hypoglycemia and hypotonia. The *SUCLA2* and *SUCLG1* genes encode the alpha and beta subunits, respectively, of the succinate-CoA ligase enzyme that catalyzes the conversion of succinyl-CoA and ADP/GDP to succinate and ATP/GTP in the TCA cycle. Patients with deficiency of succinyl-CoA ligase have been described with encephalopathy with mitochondrial DNA depletion and methylmalonic aciduria. The *AMN* and *CUBN* genes both encode gene products that form a complex that acts as a receptor for vitamin B_{12} and gastric intrinsic factor. Pathogenic variants in these genes are associated with megaloblastic anemia due to vitamin B_{12} deficiency.

Genetics:
All of the disorders tested for in this panel are inherited in an autosomal recessive manner with the exception of methylmalonic academia and homocysteinemia, cblX type (HCFC1 gene) which is X-linked.

Test Methods:
Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size.

Clinical Sensitivity:
In patients with methylmalonic acidemia and/or hyperhomocysteinemia/homocystinuria due to variant(s) in one of the genes on this panel, it is estimated that this test would detect two variants in approximately 89-95% of patients.[7, 8, 9, 10, 12]

Gene	Associated Disorder(s)
ABCD4	Methylmalonic aciduria and homocystinuria, cblJ type
ACSF3	Combined malonic and methylmalonic aciduria
AMN	Megaloblastic anemia-1, Norwegian type
CD320	Transcobalamin II receptor defect
CUBN	Megaloblastic anemia-1, Finnish type
HCFC1	Methylmalonic acidemia and homocysteinemia, cblX type

Gene	Associated Disorder(s)
LMBRD1	CblF deficiency
MCEE	Methylmalonyl-CoA epimerase deficiency
MLYCD	Malonyl-CoA decarboxylase deficiency
MMAA	Methylmalonic acidemia; CblA deficiency
MMAB	Methylmalonic acidemia; CblB deficiency
MMACHC	Methylmalonic acidemia and homocystinuria; CblC deficiency
MMADHC (C20ORF25)	CblD deficiency
MTR	CblG deficiency
MTRR	CblE deficiency
MUT	Methylmalonic acidemia mutase deficiency
SUCLG1	Encephalopathy with mitochondrial DNA depletion and methylmalonic aciduria
SUCLA2	Encephalopathy with mitochondrial DNA depletion and methylmalonic aciduria
TCN2	Transcobalamin II deficiency

References:

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