

## *HADHA* and *HADHB* Gene Analysis in Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)/ Mitochondrial Trifunctional Protein (MTP) Deficiency

### **Clinical Features:**

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency and mitochondrial trifunctional protein deficiency (MTP) deficiency also known as trifunctional protein (TFP) deficiency are disorders due to different defects in the mitochondrial trifunctional protein (MTP). MTP is an enzyme complex at the inner mitochondrial membrane with three enzymatic activities: long-chain 3-hydroxyacyl-CoA dehydrogenase, 2-enoyl hydratase, and 3-keto acyl-CoA thiolase activities. Both isolated LCHAD deficiency and MTP deficiency, which has deficiencies in all 3 enzyme activities, have overlapping clinical presentations. The variable presentation includes infantile hypoketotic hypoglycemia, vomiting, lethargy, hypotonia, and failure to thrive. Additional presentations are cardiomyopathy and cardiac conduction defects, severe liver disease, recurrent muscle cramps, seizures, coma or sudden death (SIDS)<sup>2</sup>. Peripheral neuromyopathy, and pigmentary retinopathy may develop at a later age.<sup>2</sup> The high mortality rate for these disorders, estimated at 38%,<sup>4</sup> is usually due to a Reye-like illness or fatal cardiomyopathy. Syndromes of maternal illness, HELLP (hypertension, elevated liver enzymes, low platelets) and AFLP (acute fatty liver of pregnancy) may occur in a pregnancy carrying a fetus with LCHAD/MTP deficiency.<sup>4</sup>

### **Genetics:**

Mitochondrial trifunctional protein (MTP) catalyzes the last three steps of the  $\beta$ -oxidation of long-chain fatty acids. The enzyme complex is an octomer with 4 alpha and 4 beta subunits. The  $\alpha$ -subunit is encoded by the *HADHA* gene, while the  $\beta$ -subunit is encoded by the *HADHB* gene. Both genes are located on chromosome 2p23. Biochemically, isolated LCHAD deficiency refers to a reduction in the enzymatic activity of LCHAD, while MTP deficiency has a reduction in all three enzyme activities of the MTP.<sup>1</sup> Most individuals with defects in MTP have isolated LCHAD deficiency that is due to variants in the *HADHA* gene. Less commonly, individuals are identified with defects in all three MTP activities; these can be due to variants in *HADHA* or *HADHB*. Even more rare, variants in *HADHB* that cause isolated 3-keto acyl-CoA thiolase deficiency can be observed<sup>5</sup>. Variants in these genes cause the accumulation of long-chain fatty acids and their metabolites. These metabolites are detectable in body fluids of individuals with MTP and LCHAD deficiencies and although newborn screening for these disorders is done in many states, some cases of LCHAD/MTP deficiency are not detected by newborn screening. Confirmation of test findings can be done by molecular analysis of the *HADHA* and *HADHB* genes. The frequency of isolated LCHAD deficiency has not been determined, and MTP deficiency is even less common.

**Inheritance Pattern:**

Autosomal Recessive

**Test Methods:**

Variant analysis of the *HADHA* gene is performed on genomic DNA from the submitted specimen. GeneDx offers the option of first testing for the common LCHAD deficiency *HADHA* variant (c.1528 G>C) by restriction enzyme digestion. This variant is found in approximately 60-87% of alleles.<sup>6</sup> This test can be ordered alone or with reflex full sequence analysis of the *HADHA* gene if a homozygous variant is not observed. For full sequencing the entire coding sequence and intron/exon boundaries of the 20-exon *HADHA* gene is analyzed by bi-directional sequencing. If two variants are not identified in the *HADHA* gene, sequencing of the *HADHB* gene (exons 1-16) can be performed as a reflex test upon request; alternatively, testing can be ordered simultaneously. Variants first identified in a family are confirmed by repeat analysis using sequencing, restriction fragment analysis, or another appropriate method.

**Test Sensitivity:**

In one study in patients with abnormal fibroblast LCHAD enzyme activity, 23/24 individuals were found to have two variants the *HADHA* gene; one patient had only a single gene variant identified.<sup>4</sup> In another study of 52 French, MTP-deficient patients diagnosed by either increased plasma 3-hydroxy long-chain acylcarnitines, abnormal palmitate/myristate oxidation assays, or reduced fibroblast LCHAD activities, two variants in either the *HADHA* or *HADHB* genes were identified in all patients.<sup>6</sup> The methods used by GeneDx are expected to be greater than 99% sensitive at detecting variants identifiable by sequencing.

**Variant Spectrum:**

A common variant in the *HADHA* gene (c.1528 G>C) in exon 15, accounts for approximately 87% of alleles in isolated LCHAD deficiency.<sup>3,4</sup> Variants in *HADHA* are mostly missense, nonsense, small insertions/deletions, gross deletions, splice site changes, and frameshift. The majority of variants identified in *HADHB* are missense, nonsense, small insertions/deletions, and frameshift, although gross deletions have also been reported.

**References:**

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