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)[2]. Peripheral neuromyopathy, recurrent rhabdomyolysis, and pigmentary retinopathy may develop at a later age.[2,7,8] The high mortality rate for these disorders, estimated at 38%,[4] is usually due to a Reye-like illness or fatal cardiomyopathy. Syndromes of maternal illness, AFLP (acute fatty liver of pregnancy), HELLP (hypertension, elevated liver enzymes, low platelets) syndrome, may occur in a pregnancy carrying a fetus with LCHAD/MTP deficiency.[4]

GENETICS

Mitochondrial trifunctional protein (MTP) catalyzes the last three steps of the β-oxidation of long-chain fatty acids. The enzyme complex is an octomer with 4 alpha and 4 beta subunits. The α-subunit is encoded by the HADHA gene, while the β-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.[1] 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[5]. 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

Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the HADHA and HADHB genes 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 the reference sequence 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. Testing for the HADHA and HADHB genes can be ordered sequentially, if specifically requested, or both genes can be analyzed simultaneously if a more rapid turnaround time is needed.

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.

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. [3,4] 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.

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