BCKDHA, *BCKDHB* and *DBT* Gene Analysis in Maple Syrup Urine Disease (MSUD) or *DLD* Gene Analysis in MSUD Type 3 (Dihydrolipoamide Dehydrogenase Deficiency)

Clinical Features

Maple syrup urine disease (MSUD) is a disorder of branched chain amino acid metabolism that is often classified by clinical phenotype as classic, intermediate or intermittent. The classic form presents as a neonate with the ingestion of dietary protein, with a maple syrup-like odor of cerumen, sweat and urine, irritability, poor feeding, vomiting, lethargy, and a progressive encephalopathy that may result in coma with respiratory failure. The intermediate and intermittent forms are milder and may present with anorexia, poor growth, irritability or developmental delay later in infancy or childhood, frequently in response to stress; however, even patients who are without symptoms can develop a sudden life-threatening coma. The intermittent form presents with encephalopathy and metabolic disturbances when the patient undergoes catabolic stress.[1]

The clinical phenotype of dihydrolipoamide dehydrogenase deficiency (DLDD) differs considerably from that seen in classic, intermediate or intermittent MSUD and ranges from severe neonatal presentation with neurological deficits to less severe presentations in childhood that include exertional fatigue between decompensation episodes. Individuals may also present with severe liver failure.[2] Testing for DLDD is offered separately from that for MSUD.

Genetics

MSUD is caused by decreased activity of the branched-chain alpha-ketoacid dehydrogenase complex (BCKDC), the second enzymatic step in the degradation of branched-chain amino acids leucine, isoleucine and valine. BCKDC is composed of two alpha (E1α) and two beta (E1β) subunits, a dihydrolipoyl transacylase (E2), and a dihydrolipoamide dehydrogenase (E3). Deficiency of the E1α, E1β or E2 subunits result in MSUD. The E3 subunit is shared with pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase complexes and deficiency of this subunit results in a phenotype that differs considerably from that seen in classic, intermediate or intermittent MSUD.[1]

During episodes of metabolic crisis, the plasma amino acid profiles of patients with MSUD show elevations of leucine; isoleucine and valine may also be elevated. Alloisoleucine, a distinctive metabolite of leucine, is often present and is virtually diagnostic for MSUD. Urinary organic acids show accumulation of branched-chain ketoacids. The E1α, E1β and E2 subunits are encoded by the *BCKDHA, BCKDHB* and *DBT* genes, respectively. *BCKDHA* is located on chromosome 19q13.1- q13.2 and has 9 exons. *BCKDHB* is located on chromosome 6q14 and has 11 exons. *DBT* is located on chromosome 1p31 and has 11 exons.

DLDD is caused by decreased activity of dihydrolipoamide dehydrogenase (E3). The E3 protein is a component of multiple enzyme complexes, including BCKDC; therefore, deficiency of E3 results in extensive metabolic disturbances including lactic acidemia, Krebs cycle dysfunction, and impaired branch-chain amino acid metabolism. Due to the accumulation of branched-chain amino acids in most patients with E3 deficiency, it has often been classified as a variant form of MSUD. The E3 subunit is coded by the *DLD* gene. *DLD* is located on chromosome 7q31-q32 and has 14 exons.

Inheritance Pattern

Autosomal Recessive

T: 1 (844) 241-1233 | **F:** 1 (201) 421-2020 | GeneDx.com
207 Perry Parkway | Gaithersburg, MD 20877

Test Methods

Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the *BCKDHA, BCKDHB, DBT* genes or the *DLD* gene 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 *BCKDHA, BCKDHB* and *DBT* genes can be ordered sequentially, if specifically requested, or all 3 genes can be analyzed simultaneously if a more rapid turnaround time is needed. Analysis of the *DLD* gene is available as a separate test.

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

In patients with MSUD, 45% have variants in the *BCKDHA* gene, 35% have variants in the *BCKDHB* gene, and 20% have variants in the *DBT* gene.[1] Missense, nonsense, splice-site, small deletions/insertions, and gross deletions have been reported in all three genes.[3] Most individuals are compound heterozygotes for rare sequence variants although certain variants are common in specific ethnic groups including the c.1312 T>A (Y438N) variant in the *BCKDHA* gene in Old Order Mennonites of southeastern Pennsylvania. Genotype/phenotype correlations have not been well defined in MSUD.

Variants in the *DLD* gene consist of missense, splicing, and small deletions/insertions.[3] In patients of Ashkenazi Jewish ancestry, the G229C missense variant was identified on 12 of 14 mutant alleles and is associated with a carrier frequency in this population of 1 in 94.4 Patients who are homozygous for G229C were reported to have a milder, late-onset DLD with liver failure and no neurological symptoms.[4] Apart from G229C, most individuals are compound heterozygotes for private variants therefore genotype/phenotype correlations are unclear at present.[5]

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