

BCKDHA, *BCKDHB* and *DBT* Gene Analysis in Maple Syrup Urine Disease (MSUD) and *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.

The clinical phenotype of Dihydrolipoamide Dehydrogenase Deficiency (DLD) 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. Patients may also present with severe liver failure.

Genetics:

MSUD is caused by decreased activity of the branched-chain alpha-ketoacid dehydrogenase complex (BCKAD), the second enzymatic step in the degradation of branched-chain amino acids leucine, isoleucine and valine. The BCKAD is composed of two alpha (E1a) and two beta (E1b) subunits, a dihydrolipoyl transacylase (E2) and a dihydrolipoamide dehydrogenase (E3). Deficiency of the E1a, E1b or E2 subunits result in MSUD. The E3 subunit is shared with pyruvate 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. 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 usually present and is virtually diagnostic for MSUD. Urinary organic acids show accumulation of branched-chain ketoacids. The E1 α , E1 β 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.

DLD is caused by decreased activity of dihydrolipoamide dehydrogenase (E3). The E3 subunit is a component of multiple enzyme complexes; 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

Test Methods:

In patients with MSUD, variant analysis of the *BCKDHA*, *BCKDHB* and *DBT* genes is performed on genomic DNA from the submitted specimen using bi-directional sequence analysis of the coding exons and the corresponding intron/exon boundaries. If clinically indicated, for patients who have a single variant identified after full sequencing of all three genes, or when otherwise appropriate, GeneDx will perform reflex deletion/duplication testing (ExonArrayDx) of the appropriate gene at no additional charge. Variants found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis, or another appropriate method. 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. If other studies have determined which subunit of the complex is defective, sequencing of the appropriate gene should be ordered.

In patients with DLD, variant analysis of the *DLD* gene is performed on genomic DNA from the submitted specimen using bi-directional sequence analysis of coding exons and corresponding exon/intron boundaries. If sequencing identifies a variant on only one allele of the *DLD* gene, and if clinically indicated, reflex deletion/duplication testing (ExonArrayDx) will be performed at no additional charge to evaluate for a deletion/duplication of one or more exons of this gene. Variants found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis or another appropriate method.

Test Sensitivity:

In patients with MSUD, variant analysis of the *BCKDHA*, *BCKDHB* and *DBT* genes is expected to identify variants on approximately 95% of alleles.¹⁻³ In 33 patients in which complementation studies were performed in order to determine which subunit was deficient, variants were found on 20/20 *BCKDHA* alleles, on 29/30 *BCKDHB* alleles, and on 13/16 *DBT* alleles.⁴ In a series of small studies of 15 patients with deficiency of the dihydrolipoamide dehydrogenase enzyme, variants in the *DLD* gene were identified on 30/30 alleles.⁵⁻¹²

The methods used by GeneDx are expected to be greater than 99% sensitive at detecting variants identifiable by sequencing.

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.¹³ Missense, nonsense, splice-site, small deletions/insertions, and gross deletions have been reported in all three genes.¹⁴ 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) 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. 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 94.⁵ Patients who are homozygous for G229C were reported to have a milder, late-onset DLD with liver failure and no neurological symptoms.⁵ Apart from G229C, most individuals are compound heterozygotes for private variants therefore genotype/phenotype correlations are unclear at present.⁸

References:

1. Quental et al. (2008) *Molecular Genetics And Metabolism* 94 (2):148-56 (PMID: 18378174)
2. Flaschker et al. (2007) *Journal Of Inherited Metabolic Disease* 30 (6):903-9 (PMID: 17922217)
3. Nellis et al. (2003) *Molecular Genetics And Metabolism* 80 (1-2):189-95 (PMID: 14567968)
4. Rodriguez-Pombo et al. (2006) *Human Mutation* 27 (7):715 (PMID: 16786533)
5. Shaag et al. (1999) *Am. J. Med. Genet.* 82 (2):177-82 (PMID: 9934985)
6. Odievre et al. (2005) *Hum. Mutat.* 25 (3):323-4 (PMID: 15712224)
7. Grafakou et al. (2003) *Eur. J. Pediatr.* 162 (10):714-8 (PMID: 12925875)
8. Cameron et al. (2006) *American Journal Of Medical Genetics. Part A* 140 (14):1542-52 (PMID: 16770810)
9. Hong et al. (1996) *Hum. Mol. Genet.* 5 (12):1925-30 (PMID: 8968745)
10. Liu et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90 (11):5186-90 (PMID: 8506365)
11. Shany et al. (1999) *Biochem. Biophys. Res. Commun.* 262 (1):163-6 (PMID: 10448086)
12. Hong et al. (1997) *Biochim. Biophys. Acta* 1362 (2-3):160-8 (PMID: 9540846)
13. Strauss KA, Puffenberger EG, Morton DH. Maple Syrup Urine Disease. 2006 Jan 30 [Updated 2013 May 9]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. *GeneReviews*® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1319/>
14. Stenson et al. (2014) *Human Genetics* 133 (1):1-9 (PMID: 24077912)