THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Genetic Testing for D-2-Hydroxyglutaric Aciduria

Clinical Features:

D-2-hydroxyglutaric aciduria (D2HGA) type 1 [OMIM #600721] and type 2 [OMIM#613657] are rare neurometabolic diseases associated with accumulation of D-2-hydroxyglutaric aciduria (D-2-HG) in urine (1). The cardinal clinical manifestations in both D2HGA subtypes are developmental delay, hypotonia and seizures (1). Age of onset is similar for both subtypes, typically occurring at 0-6 months of age in D2HGA type 1, and 0-2 years of age in D2HGA type 2. Additional clinical features described in some patients with D2HGA include macrocephaly, dysmorphic features and cerebral visual abnormalities. 47% of D2HGA type 2 patients have cardiomyopathy (primarily dilated). Brain MRI findings in D2HGA patients include enlargement of lateral ventricles, enlarged subarachnoid spaces, subdural effusions, subependymal psuedocysts, signs of delayed cerebral maturation and multifocal cerebral white matter abnormalities. Differences between MRI findings patients with D2HGA type 1 compared to type 2 have not been delineated to date.

L-2-hydroxyglutaric aciduria (L2HGA) [OMIM #147920] is associated with accumulation of L-2-hydroxyglutaric aciduria in urine (1). L2HGA is a slowly progressive disorder with average age of onset of 2 years (range 0-7). The most commonly observed clinical features are developmental delay, epilepsy and cerebellar ataxia (2). Other features that can be observed in some patients include macrocephaly, hypotonia, extrapyramidal signs, behavioral problems and spasticity (2). Patients typically have a characteristic pattern of findings on brain MRI, including subcortical cerebral white matter abnormalities, signal intensities of the caudate nucleus, putamen, dentate nucleus and globus pallidus, and atrophy of the cerebral white matter, cerebellar vermis and hemispheres (3).

Affected individuals with combined D-2 and L-2-hydroxyglutaric aciduria (D,L-2-HGA) [OMIM #615182] typically have severe neonatal epileptic encephalopathy and absence of developmental progress (4). Death in the first year of life is common. Enlarged ventricles, subependymal pseudocysts and delayed gyration and myelination are typical brain MRI findings in affected patients (1).

Molecular and Biochemical Genetics:

Conventional urine organic acid screening with gas chromatography mass spectrometry (GC-MS) can detect increased 2-HG (2-hydroxyglutaric acid), but does not differentiate between enantiomeric D-2-HG and L-2-HG (1).

Mutations in the *D2HGDH* [OMIM #609186] gene are associate with D2HGA type 1, and mutations in the *IDH2* gene [OMIM #147650] gene are associated with D2HGA type 2 (1). Mutations in either gene are associated with accumulation of D-2-HG in the urine and cerebral spinal fluid. *D2HGDH* encodes D-2-hydroglutarate dehydrogenase, which converts D-2-HG to 2-ketoglutaric acid. Loss of function mutations in *D2HGDH* lead to an accumulation of D-2-HG. To date, missense, nonsense, splice site and frameshift mutations in *D2HGDH* have been described. *IDH2* encodes isocitrate dehydrogenase-2, which normally converts isocitrate to 2-ketoglutaric aciduria. Mutations in *IDH2* associated with D2HGA are gain-of-function missense mutations, which give isocitrate dehydrogenase-2 the ability to convert 2-ketoglutaric aciduria to D-2-HG. This causes D-2-HG to accumulate.

Mutations of the *L2HGDH* gene [OMIM #609584] gene are associated with L2HGA. Missense, nonsense, frameshift and splice site mutations have been described. The *L2HGDH* gene encodes for the enzyme L-2-hydroxyglutarate dehydrogenase (L-2-HGDH). Typically, L-2-hydroxyglutaric acid (L-2-HG) is converted to 2-ketoglutarate (2-KG) by L-2-HGDH. Mutations in *L2HGDH* are associated with accumulation of L-2-HG in the urine and cerebral spinal fluid.

Homozygous and compound heterozygous mutations of the *SLC25A1* gene [OMIM #190315] gene are associated with D,L-2-HGA (4). Missense, nonsense and frameshift mutations have been described. The *SLC25A1* gene plays a role transporting citrate from the mitochondria to the cytosol, where it is converted into acetyl coenzyme A. Acetyl coenzyme-A is essential for fatty acid and sterol synthesis. Individuals with D,L-2-HGA have increased levels of both D-2 hydroxyglutaric acid and L-2 hydroxyglutaric acid in the urine. It is hypothesized that the increased excretion of these two compounds is related to impaired citrate transport from the mitochondria and disruption of the Krebs cycle caused by *SLC25A1* mutations (4).

Inheritance:

D2HGDH, L2HGDH and *SLC25A1* mutations follow an autosomal recessive inheritance pattern. Parents of an affected child are most likely obligate carriers. Recurrence risk for carrier parents is 25%. Mutations in *IDH2* are inherited in an autosomal dominant manner. *IDH2* mutations are typically *de novo*, and recurrence risk is therefore low. A case of germline mosaicism for an *IDH2* mutation has been reported (1).

Test methods:

We offer full gene sequencing of all coding exons and intron/exon boundaries of *D2HGDH, IDH2, L2HGDH* and *SLC25A1*. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. The constructed genomic DNA library is sequenced using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20bp.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

D-2- and L-2 Hydroxyglutaric Aciduria Panel (*D2HGDH, IDH2, L2HGDH* and *SLC25A1* sequence and del/dup analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$1500
CPT codes:	81406 81407
Turn-around time:	8 weeks

D-2-Hydroxyglutaric Aciduria Panel (*D2HGDH* and *IDH2* sequence and del/dup analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$1500
CPT codes:	81406 81407
Turn-around time:	4 weeks

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

1. Kranendijk M, Struys EA, Salomons GS et al. Progress in understanding 2-hydroxyglutaric acidurias. J Inherit Metab Dis 2012: 35: 571-587.
2. Steenweg ME, Jakobs C, Errami A et al. An overview of L-2-hydroxyglutarate dehydrogenase gene (L2HGDH) variants: a genotype-phenotype study. Hum Mutat 2010: 31: 380-390.
3. Steenweg ME, Salomons GS, Yapici Z et al. L-2-Hydroxyglutaric aciduria: pattern of MR imaging abnormalities in 56 patients. Radiology 2009: 251: 856-865.
4. Nota B, Struys EA, Pop A et al. Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. Am J Hum Genet 2013: 92: 627-631.

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