ETFA, ETFB, and ETFDH Gene Analysis in Glutaric Aciduria II (GAII) or Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)

CLINICAL FEATURES

Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) or Glutaric Aciduria II (GAII)is a rare disorder thatcanresult from deficiency of the alpha or beta subunits of electron transfer flavoprotein or its dehydrogenase ETF:ubiqinone oxidoreductase (ETF-DH or ETF:QO). Defects in any of these genes may lead to a range of clinical phenotypes from mild to severe, depending upon the pathogenic variant. Three clinical phenotypes for GAII have been described. Type I presents as a life-threatening disorder during the neonatal period with tachypnea, dyspnea, profound acidosis, severe hypotonia, and convulsions. Hepatomegaly, hypoketotic hypoglycemia, hyperammonemia, sweaty-sock like odor, and congenital anomalies including renal cystic dysplasia, heart abnormalities, central nervous system malformations, facial dysmorphism, rocker bottom feet, and abnormalities of the external genitalia may also be present. Type II presentation is similar to Type I without congenital anomalies, while Type III occurs later with intermittent episodes of vomiting, hypoglycemia, and metabolic acidosis during infancy or episodic muscular weakness and pain during adulthood along with progressive myopathy; this type may be underdiagnosed.9 Recurrent pancreatitis has also been reported with abdominal symptoms.[1,2]

GENETICS

The electron transfer flavoprotein (ETF) is located in the mitochondrial matrix as a heterodimer of alpha and beta subunits and electron transfer flavoprotein dehydrogenase (ETF-DH) is located in the inner mitochondrial membrane. ETF is an electron acceptor for the acyl-CoA dehydrogenases involved in fatty acid oxidation as well as for several dehydrogenases involved in amino acid and choline metabolism. These electrons are subsequently transferred via ETF-DH to ubiquinone in the respiratory chain. During episodes of metabolic crisis, urinary organic acid profiles show dicarboxylic aciduria and a characteristic accumulation of marker metabolites of the blocked dehydrogenases. Plasma acylcarnitine analysis shows an increase of all chain length acylcarnitines. Patients with GAII may have normal organic acid profiles between periods of catabolic stress. The two ETF subunits are encoded by the ETFA (12 exons) and ETFB(6 exons) genes located on chromosomes 15q23-25 and 19q13.3, respectively. ETF-DH is encoded by the ETFDH gene (13 exons) on chromosome 4q32-qter. As not all patients with GAII have pathogenic variants in the ETFA, ETFB or ETFDH genes, pathogenic variants in other genes are predicted to also cause GAII.[1,3,4,5] Recently, defects in genes encoding riboflavin transporters have been identified that can result in similar biochemical and clinical abnormalities.9 Of patients with variants identified in ETFA, ETFB or ETFDH, ~11-27% of patients had ETFAvariants, ~27-33% had ETFB variants, and ~47-56% had ETFDH variants.[3, 6] Of 350 patients with the Type III presentation, 93% had variants in the ETFDH gene, while variants in ETFA (5%) and ETFB (2%) were rare.9 Most patients with riboflavin-responsive GAII have been reported to have pathogenic variants in the ETFDHgene.[7, 8] Of patients with the Type III presentation, 98.4% were riboflavin-responsive.[9]

INHERITANCE PATTERN

Autosomal Recessive

TEST METHODS

Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the ETFDH, ETFB, and ETFAgenes 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 ETFDH, ETFB, and ETFAgenes can be ordered sequentially, if specifically requested, or each gene 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

Variants in the three genes include missense, nonsense, frameshift, gross deletions, small insertions and deletions, frameshift, and splice site changes.[3]

REFERENCES:

1. Schiff, M, et al. (2006) Mol Genet Metab 88(2):153- 158.
2. Gordon, N. (2006) Brain Dev 28(3):136-140.
3. Olsen, RKJ, et al (2003) Hum Mutat 22:12-23.
4. Spaan, AN, et al. (2005) Mol Genet Metab 86(14):441-447.
5. Goodman, SI, et al. (2002) Mol Genet Metab 77(1-2):86-90.
6. Yotsumoto et al., (2008) Mol Genet Metab 94:61-67.
7. Olsen, RKJ, et al., (2007) Brain 130:2045-2054.
8. Wen et al., (2010) J Neurosurg Psychiatry 81:231-236.
9. Grunert, S. (2014) Orphanet Journal of Rare Diseases 9:117.