Elevated C4 Acylcarnitine Panel

Panel Gene List: *ACAD8, ACADS, ETHE1, ETFA, ETFB, ETFDH*

Indication for Testing:
Identification of elevated blood butyrylcarnitine/isobutyrylcarnitne (C4 acylcarnitine) on biochemical analysis, such as a positive newborn screen or plasma acylcarnitines. Genetic testing of these 6 genes may confirm a diagnosis, determine appropriate monitoring or treatment, and facilitate family studies.

Introduction:
Mitochondrial fatty acid oxidation is the principal pathway for energy production during times of increased energy demand such as prolonged fasting, illness, and exercise. During fasting when glucose supplies are low, long-chain fatty acids stored as triglycerides in fat tissue are released by lipases and activated to acyl-CoA esters that are transported into the mitochondria via the carnitine shuttle. Once inside the mitochondria, several chain-length specific enzymes shorten acyl-CoAs, two carbon atoms at a time, via β-oxidation cycles. Long-chain compounds are metabolized at the inner mitochondrial membrane, while medium and short-chain compounds are metabolized in the mitochondrial matrix. Defects in this process result in disorders of fatty acid oxidation.

Clinical Features:
A subset of fatty acid oxidation disorders are detectable by newborn screening due to elevated blood C4 acylcarnitine concentrations, with potential treatment implications. These disorders include ethylmalonic encephalopathy (EE), multiple acyl-CoA dehydrogenase (MADD) deficiency or glutaric acidemia types IIA, IIB, and IIC (GAII), isobutyryl-CoA dehydrogenase (IBD) deficiency, and short chain Acyl-CoA dehydrogenase (SCAD) deficiency. IBD deficiency and SCAD deficiency have a biochemical phenotype of elevated butyrylcarnitine/isobutyrylcarnitine (C4 acylcarnitine) typically ascertained by a newborn screening, although most individuals identified with these disorders do not have an associated clinical phenotype.[1]

Ethylmalonic Encephalopathy (EE)
The clinical manifestations of EE include psychomotor regression and generalized hypotonia which progresses into spastic tetraparesis, dystonia, and global neurological failure. MRI shows necrotic lesions in the basal ganglia and brainstem. The encephalopathy is typically accompanied by petechia and orthostatic acrocyanosis. Chronic diarrhea is also common. EE has the highest incidence in individuals from the Mediterranean basin or Arabic peninsula.[6]

Multiple Acyl-CoA Dehydrogenase (MADD) Deficiency/Glutaric Acidemia types IIA, IIB, and IIC (GAII)
GAII 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 (cystic dysplasia, heart abnormalities, central nervous system malformations, facial dysmorphism, rocker bottom feet, abnormalities of the external genitalia) may also be present. Type II is similar to Type I without the congenital anomalies, and Type III has later onset with intermittent episodes of vomiting, hypoglycemia, and metabolic acidosis during infancy or episodic muscular weakness and pain and progressive myopathy during adulthood.

Individuals with MADD may have elevated C4 acylcarnitines and/or C5 acylcarnitines detected via newborn screening.[7] Other biochemical findings include abnormal urine organic acids with increased ethylmalonic and 2-methylsuccinic acid, metabolic acidosis, and hyperammonemia.[8] Some individuals only show biochemical abnormalities under metabolic stress.[1] Genetic testing can confirm the diagnosis and in some cases genotype/phenotype correlations are possible.

Isobutyryl-CoA Dehydrogenase (IBD) Deficiency
IBD deficiency was first reported in a child with dilated cardiomyopathy, anemia and secondary carnitine deficiency.[2] Very few patients have been reported with clinical findings attributable to IBD deficiency. The majority of reported individuals with IBD deficiency have been identified after the detection of elevated C4-carnitine by tandem mass spectrometry based newborn screening. Patients first identified by screening have either remained asymptomatic or presented with milder clinical phenotypes including muscle hypotonia, and mild developmental delay.

Short Chain Acyl-CoA Dehydrogenase (SCAD) Deficiency
The clinical phenotype attributed to SCAD deficiency in early studies included one or more of the following: developmental delay, seizures, hypotonia, hypoglycemia, and/or failure to thrive. Some individuals have been identified later in childhood or as adults, with muscle weakness or progressive myopathy but it is not clear that SCAD deficiency was the explanation for the phenotype.[3,4] In a large study, 31 patients were followed after being diagnosed subsequent to a positive newborn screening result. Twenty-two of these patients had sequencing of the *ACADS* gene. No patients had epilepsy but hypoglycemia, speech delay, hypotonia central apnea or poor feeding were identified in 6 patients, although these clinical findings were thought to have explanations other than SCAD deficiency.[5] The vast majority of infants detected by newborn screening remain asymptomatic.[1]

Genetics:
Disorders involving *ACAD8, ACADS, ETHE1, ETFA, ETFB*, and *ETFDH* are inherited in an autosomal recessive manner.

Test Methods:
Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested 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 reference sequences 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.

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.

References:

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