Genetic Testing for Hyperammonemia: Urea Cycle Disorders, Transporter Defects and Other Disorders Associated with Elevated Ammonia

Panel Gene List: *ACADM, ACADVL, ARG1, ASL, ASS1, BCKDHA, BCKDHB, CA5A, CPS1, CPT1A, CPT2, DBT, DLD, ETFA, ETFB, ETFDH, GLUD1, HADHA, HADHB, HCFC1, HLCS, HMGCL, HMGCS2, IVD, MCCC1, MCCC2, MMAA, MMAB, MMACHC, MMADHC (C2ORF25), MUT, NAGS, OAT, OTC, PC, PCCA, PCCB, PDHA1, PIGA, SERAC1, SLC22A5, SLC25A13, SLC25A15, SLC25A20, SLC7A7, SUCLA2, SUCLG1, TMEM70*

Clinical Features:

The presence of hyperammonemia in plasma is indicative of an overproduction of ammonia or a failure in ammonia detoxification. The hepatic urea cycle is the main pathway for ammonia detoxification. Primary hyperammonemia can be due to inherited deficiencies of one of the enzymes in the urea cycle pathway or in one of the two membrane transporters. Secondary hyperammonemia may be caused by inherited defects in related pathways that are necessary for the production of ammonia acceptors or other substrates necessary for the proper function of the urea cycle.[1]

Hyperammonemic disorders that are not due to general liver failure are rare, they can affect patients at any age and they are usually associated with non-specific neurological symptoms that vary depending upon the age of the patient and the type and severity of the underlying disorder. Hyperammonemic newborns usually present with poor feeding, vomiting, seizures, unstable body temperature, respiratory distress or poor peripheral blood circulation, while children and adults present with vomiting, ataxia, confusion, disorientation, hallucinations or abnormal behavior.

Primary Hyperammonemia: Urea Cycle Disorders and Transporter Defects:

The six enzymes of the urea cycle are carbamoylphosphate synthetase I (CPS1), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (ASS1), argininosuccinic acid lyase (ASL), arginase (ARG1), and the cofactor producing enzyme: N-acetyl glutamate synthetase (NAGS). In addition to the six enzymes, two transporters are also necessary for the proper function of the urea cycle: the ORNT1 transporter, ornithine translocase (SLC25A15) and citrin (SLC25A13). Deficiencies in any of these enzymes or transporters can result in hyperammonemia and disordered amino acid metabolism. The disease presentation is highly dependent upon which protein is deficient and the severity of the deficiency. Patients with a severe deficiency of CPS1, OTC, ASS1, ASL or NAGS appear normal at birth, but accumulate ammonia during the first few days of life and, therefore, rapidly develop cerebral edema, lethargy, anorexia, hyper- or hypoventilation, hypothermia, seizures, neurologic posturing, and

coma.[2] In cases of milder enzyme deficiencies and in ARG deficiency, ammonia may accumulate during times of illness or stress at any age, and symptoms may be attenuated.[2] Deficiency of the citrin transporter results in one of two phenotypes: citrullinemia type II (CTLN2) and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). CTLN2 typically presents in adulthood with recurring neuropsychiatric symptoms associated with episodic hyperammonemia, seizures, and coma that can lead to death from brain edema, while the symptoms of NICCD are milder and typically present in children under one year of age as transient intrahepatic cholestasis, hypoproteinemia, growth retardation, hypoglycemia, fatty liver and mild liver dysfunction. Deficiency of the ORNT1 transporter results in hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome with symptoms resulting from hyperammonemia and resembling those of a urea cycle disorder.[2] The prevalence of urea cycle disorders in the United States is estimated at 1 in 8,200.[3]

A plasma ammonia concentration of 150 µmol/L or higher associated with a normal anion gap and normal plasma glucose concentration is highly suspicious of a urea cycle disorder.[2] Plasma amino acid analysis followed by, when appropriate, other biochemical tests including urinary orotic acid analysis often leads to a diagnosis of a specific urea cycle disorder or transporter defect, which can be confirmed by molecular testing or enzymatic testing. However, sometimes the specific defect is not obvious after biochemical testing. Enzymatic testing may enable a diagnosis in this situation; however, for CPS1, OTC and NAGS deficiencies this requires a liver biopsy.

Secondary Hyperammonemia: Other Inborn Errors of Metabolism

Secondary hyperammonemia may be caused by inborn errors of metabolism that inhibit the urea cycle or cause deficiency of substrates that are necessary for the proper functioning of the urea cycle due to the accumulation of toxic metabolites. For example, certain metabolites can impair the synthesis of NAGS, while some organic acids inhibit the mitochondrial Krebs cycle resulting in the reduction of α-ketoglutarate as an ammonia acceptor for glutamate synthesis.[1] Furthermore, deficiency of acetyl-CoA or glutamate in the mitochondria can lead to secondary hyperammonemia.[1]

Many of the inborn errors of metabolism that are associated with secondary hyperammonemia can be diagnosed through biochemical and/or enzymatic testing. However, biochemical testing may not always be conclusive and some enzyme assays require biopsies.

Genetics:

All of the disorders tested for in this panel are inherited in an autosomal recessive manner with the exception of glutamate dehydrogenase I deficiency (*GLUD1* gene) which is autosomal dominant and OTC deficiency (*OTC* gene), methylmalonic academia and homocysteinemia, cblX type (*HCFC1* gene), pyruvate dehydrogenase E1-alpha deficiency (*PDHA1* gene) and multiple congenital anomalies-hypotonia-seizures syndrome 2 (*PIGA* gene) which are X-linked.

Test Methods:
Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the 48 genes on this panel 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. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. 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. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: *CA5A* gene, no copy number testing, *PC* gene only whole gene deletions or duplications may be detected.

Clinical Sensitivity:
Based on the sensitivity of variant analysis for the individual genes, it is estimated that this panel would detect a pathogenic variant in 80% to greater than 95% of patients with hyperammonemia due to variants in one of the 48 genes.

Gene	Disease Associations:
ACADM	Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency
ACADVL	Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency
ASL	Argininosuccinic aciduria
ASS1	Classic citrullinemia
ARG1	Arginase deficiency
BCKDHA	Maple syrup urine disease (MSUD)
BCKDHB	Maple syrup urine disease (MSUD)
CA5A	Hyperammonemia due to carbonic anhydrase VA deficiency
CPS1	Carbamoyl phosphate synthetase I deficiency
CPT1A	Carnitine palmitoyltransferase IA deficiency
CPT2	Carnitine palmitoyltransferase II (CPT2) deficiency
DBT	Maple syrup urine disease (MSUD)

DLD	Dihydrolipoamide dehydrogenase deficiency
ETFA	Glutaric aciduria II (GAII)
ETFB	Glutaric aciduria II (GAII)
ETFDH	Glutaric aciduria II (GAII)
GLUD1	Hyperinsulinism-hyperammonemia syndrome
HADHA	Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)/ Mitochondrial trifunctional protein (MTP) deficiency
HADHB	Mitochondrial trifunctional protein (MTP) deficiency
HCFC1	Methylmalonic acidemia and homocysteinemia, cblX type
HLCS	Holocarboxylase synthetase deficiency (Multiple carboxylase deficiency)
HMGCL	HMG CoA lyase deficiency (3-Hydroxy-3-methylglutaryl CoA lyase deficiency
HMGCS2	HMG-CoA synthase-2 deficiency (3-Hydroxy-3-methylglutaryl-CoA synthase 2 deficiency
IVD	Isovaleric acidemia
MCCC1	3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency
MCCC2	3-Methylcrotonyl-CoA carboxylase (3-MCC) deficiency
MMAA	Methylmalonic acidemia
MMAB	Methylmalonic acidemia
MMACHC	Methylmalonic aciduria and homocystinuria, cobalamin C (cblC) type
MMADHC (C2ORF25)	Methylmalonic aciduria and homocystinuria, cobalamin D (cblD) type
MUT	Methylmalonic acidemia
NAGS	N-acetylglutamate synthase deficiency
OAT	Gyrate atrophy of choroid and retina with or without ornithinemia
OTC	Ornithine transcarbamylase deficiency
PC	Pyruvate carboxylase deficiency
PCCA	Propionic acidemia
PCCB	Propionic acidemia
PDHA1	Pyruvate dehydrogenase E1-alpha deficiency
PIGA	Multiple congenital anomalies-hypotonia-seizures syndrome 2
SERAC1	3-Methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome
SLC22A5	Primary/systemic carnitine deficiency
SLC25A13	Citrullinemia type II (CTLN2) and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)
SLC25A15	Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome
SLC25A20	Carnitine-acylcarnitine translocase deficiency
SLC7A7	Lysinuric protein intolerance
SUCLG1	Encephalopathy with mitochondrial DNA depletion and methylmalonic aciduria

SUCLA2	Encephalopathy with mitochondrial DNA depletion and methylmalonic aciduria

References:

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4. Bennett S.(2004) Pharmacogenomics 5:433-8.