ASPA Gene Analysis in Canavan Disease

CLINICAL FEATURES

Canavan disease (CD) is a neurodegenerative leukodystrophy that typically presents as a neonatal/infantile (severe) form with onset of symptoms at 2-4 months that include poor head control, macrocephaly, truncal hypotonia, and developmental delay. Severe CD is associated with delayed motor skills and the inability for these children to sit, stand, walk or talk. Many severe patients also have optic atrophy. Over time, spasticity develops and sleep disturbance, seizures and feeding difficulties may be present. Spongy degeneration of the white matter is present, with swollen astrocytes and elongated mitochondria. The life expectancy of patients with the severe form is variable with survival reported from months to past the teen years.¹A much more rare mild/juvenile form of CD also exists that is characterized by mild developmental delay that may go unrecognized. CD occurs in all ethnic groups, but it is most common in the Ashkenazi Jewish population where the carrier rate has been estimated at 1 in 40 to 1 in 82.¹

GENETICS

CD is caused by variants in the *ASPA* gene that encodes the aspartoacylase (ASPA) enzyme that hydrolyzes Nacetyl aspartic acid (NAA) to aspartate and acetate in oligodendrocytes. Deficient ASPA activity results in the accumulation of NAA in the brain. Patients with severe CD are usually diagnosed by the presence of increased levels of NAA in urine, while in patients with mild CD NAA levels in urine may only be mildly elevated; therefore, the diagnosis of mild CD may depend upon identification of disease-causing variants in the *ASPA* gene. Measurement of ASPA enzyme activity is possible in skin fibroblasts (activity is not detectable in leukocytes) but the reliability of this test has been questioned since enzyme activity is affected by culture conditions.¹The *ASPA* gene is located on chromosome17pter-p13 and has 6 exons.

INHERITANCE PATTERN

Autosomal Recessive

TEST METHODS

Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the *ASPA* gene 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 sequence 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.



VARIANT SPECTRUM

Variants reported in the *ASPA* gene include missense, nonsense, splice site, small deletions/insertions and large deletions. In one study of 23 non-Ashkenazi Jewish patients from diverse ethnic backgrounds, large deletions that would not be detected by sequence analysis were identified in over 10% of *ASPA* alleles (5/46).²Two variants, p.Glu285Ala and p.Tyr231Stop, account for 98% of disease-causing alleles in the Ashkenazi Jewish population, and a p.Ala305Glu variant accounts for 20%-60% of disease-causing alleles in non-Ashkenazi Jewish patients.^{3, 4}Most other variants appear to be private or confined to small geographic areas.²Individuals homozygous for p.Glu285Ala, p.Tyr231Stop or p.Ala305Glu usually have a severe CD phenotype as do individuals who are either homozygous or compound heterozygous for a large deletion.1Mild CD has been associated with the presence of at least one "mild" variant: p.Tyr288Cys, p.Arg71His or p.Pro257Arg.¹

REFERENCES:

1. Matalon R, Delgado L, Michals-Matalon K. Canavan Disease. 1999 Sep 16 [Updated 2018 Sep 13]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1234/; 2.Zeng et al., (2006) Molec Genet Metab 89 :156-163

^{3.}Kaul et al., (1994) Am J Hum Genet 55:34-41 4.Zeng et al., (2002) J Inherit Metab Dis 25 :557-570