ARSA Gene Analysis in Metachromatic Leukodystrophy

CLINICAL FEATURES

Metachromatic leukodystrophy (MLD) caused by arylsulfatase A deficiency is a lysosomal storage disorder that is characterized by leukodystrophy and progressive neurologic dysfunction. Approximately 50-60% of patients have the late-infantile form with onset usually between one and two years, 20-30% of patients have the juvenile form with onset between 4 years and puberty, and 15- 20% of patients have the adult form. The late-infantile form is the most severe with loss of previously acquired skills, optic atrophy, ataxia, dementia, seizures and spastic quadriparesis. In the juvenile form, the initial symptoms are usually declining school performance and the onset of behavioral problems. Clumsiness, gait problems, slurred speech, incontinence, bizarre behavior and seizures are also observed. In some adult patients, problems in school and/or work performance, personality changes and emotional lability may be presenting symptoms, and in others neurologic symptoms including weakness, loss of coordination, seizures and peripheral neuropathy may be the first signs of disease. All forms eventually result in the complete loss of motor and intellectual functions with the disease course ranging from 3-10 years in the late-infantile form and up to 20 years in the juvenile and adult forms. Life span is usually inversely correlated with the age of onset.[1] The disease prevalence is estimated at between 1 in 40,000 to 1 in 160,000 with a higher prevalence in some populations including Habbanite Jews in Israel, Israeli Arabs, Christian Israeli Arabs and the Navajo Nation in the United States.[1]

In rare instances, patients are affected with MLD due to saposin B deficiency. Saposin B is a nonenzymatic glycoprotein necessary for the lysosomal degradation of sulfatide. Affected patients have clinical features very similar to those with MLD, but they have normal arylsulfatase A enzyme activity.

GENETICS

MLD caused by arylsulfatase A (ARSA) deficiency is due to variants in the *ARSA* gene that catalyzes the first step of the lysosomal degradation of the sphingolipid cerebroside-3-sulfate (sulfatide): a lipid mainly found in myelin. Deficiency of ARSA results in sulfatide storage that primarily affects the brain leading to progressive demyelination in the central and peripheral nervous systems. MLD is suspected if ARSA activity in leukocytes is less than 10% of normal controls; however, ARSA enzyme assays cannot distinguish between MLD and ARSA pseudodeficiency in which ARSA activity is 5- 20% of controls in an otherwise healthy individual.[1] Homozygosity for the most common ARSA pseudodeficiency allele occurs in as many as 0.5-2% of Europeans and may be more common in Asians and Africans. Therefore, a diagnosis of MLD must be confirmed by other tests, including ARSA gene analysis, urinary excretion of sulfatides and much more rarely, the presence of metachromatic lipid deposits in a nerve or brain biopsy.[1] The *ARSA* gene is located on chromosome 22q13 and has 8 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 *ARSA* 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 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.

VARIANT SPECTRUM

Variants reported in the *ARSA* gene include missense, nonsense, splice site, small deletions/insertions and large deletions.[4] Common variants exist in certain populations including four variants that occur in central and western European populations: c.459+1 G>A, c.1204+1 G>A, p.Pro426Leu and p.Ile179Ser;1 these 4 variants account for 25% to 50% of the ARSA alleles in these populations.[1] The majority of variants in the *ARSA* gene are private, and variants occur throughout the 8 exons.[5, 6] A genotype/phenotype correlation has been identified. Variants that result in no functional ARSA activity are designated as I-type (Infantile/juvenile onset) alleles; the most common Itype alleles are c.459+1 G>A and c.1204+1 G>A. Variants that are associated with some functional activity are designated as A-type (Adult onset) alleles; the most common A-type alleles are p.Pro426Ser and p.Ile179Ser. Patients with the late-infantile type of MLD are usually homozygous or compound heterozygous for I-type alleles, while patients with the later-onset forms usually have one or two A-type alleles. Juvenile-onset patients are often compound heterozygotes with an I-type allele and an A-type allele and adult-onset patients often have an A-type variant on both *ARSA* alleles.[1] Exceptions have been reported. Patients with the same genotype can have a marked difference in severity.[7]

ARSA pseudodeficiency is most commonly caused in European and American populations by two sequence variants on the same ARSA allele (in cis) designated, c.[1049 A>G, *96 A>G]. Homozygosity for the c.[1049 A>G, *96 A>G] pseudodeficiency allele occurs in approximately 0.5%- 2% of the European/Euro-American population and may be found in cis with an MLD-causing variant. [1] The c.*96 A>G variant occurs in the polyadenylation site of ARSA. The c.1049 A>G variant alters an N-glycosylation site in ARSA and is believed to reduce enzyme activity and result in decreased targeting to the lysosome.[8] Due to the common occurrence of the pseudodeficiency allele, parental testing is important to determine phase of the identified variants in the proband.

REFERENCES:

1. Arranz et al., (2002) Hum Mutat 20:180-188.
2. St-Louis, M. and Tanguay R.M. (1997) Hum Mutat 9 :291-299.
3. Grompe et al., (1994) N Engl J Med 11(6):353-7.
4. Bergman et al., (1998) Hum Mutat 12 :19-26.
5. Rootwelt et al., (1994) Am J Hum Genet 55 :1122-1127