Alagille Syndrome Panel

Disorder also known as: Alagille-Watson syndrome; Arteriohepatic Dysplasia; Cholestasis with Peripheral Pulmonary Stenosis; Syndromic Bile Duct Paucity; Syndromatic Hepatic Ductular Hypoplasia

Panel Gene List: ATP8B1, JAG1, NOTCH2*
*No sequencing or deletion/duplications analysis for exons 1-4 of NOTCH2

Clinical Features:
Alagille syndrome (ALGS) is a multi-system disorder with highly variable expressivity that is characterized by paucity of intrahepatic bile ducts, which causes chronic cholestasis, in association with cardiac, skeletal, ocular, and facial abnormalities.1,2 ALGS is one of the major forms of chronic liver disease in children and typically presents in infancy with cholestasis, which manifests as jaundice, xanthomas, and failure to thrive.2,3 The lack of intrahepatic bile ducts may or may not be demonstrable on histologic exam in the newborn period.2,5 The most common cardiac manifestation in ALGS is pulmonic stenosis, which occurs in two-thirds of patients, followed by tetralogy of Fallot, which occurs in 7-16% of patients.2,4 Other major findings include posterior embryotoxon and anterior segment abnormalities of the eyes, vertebral anomalies (“butterfly” vertebrae), and characteristic facial features (broad forehead, deep-set eyes, pointed chin, elongated nose with bulbous tip) giving the face an inverted triangular shape.5 Additionally, renal, vascular and pancreatic abnormalities are observed in a significant percentage of patients.1,5,6 Intra- and inter-familial variability has been observed. Classic clinical diagnostic criteria have been published and are based on (1) the finding of a paucity of intrahepatic bile ducts or cholestasis, and (2) the presence of three out of five characteristic findings of the liver, heart, eye, skeleton, and face, or two of these features if the patient has a positive family history.3,5 However, recent data from molecular testing for ALGS and the acknowledgment of a broader phenotypic spectrum have led to the expansion of phenotypic findings to include renal and vascular abnormalities in the diagnostic criteria.3,7

Genetics:
Alagille syndrome is an autosomal dominant disorder caused by pathogenic variants in the JAG1 and NOTCH2 genes that encode core components of the Notch signaling pathway. Rarely, ALGS-like presentation is caused by pathogenic variants in the ATP8B1 gene with an autosomal recessive mode of inheritance.

Multiple types of variants have been reported in JAG1 and NOTCH2 in association with ALGS, although large deletions/duplications, which are found in ~5-7% of ALGS cases, have only been reported in the JAG1 gene.5,8 An estimated 50-70% of individuals with ALGS due to abnormalities in JAG1 are due to de novo variants.9 Based on a small cohort study, individuals
with NOTCH2 variants present with less cardiac involvement and lower prevalence of butterfly vertebrae and facial features compared to those with JAG1 variants.\textsuperscript{10} In addition to ALGS, NOTCH2 defects cause Hajdu-Cheney syndrome (HCS), a rare autosomal dominant skeletal disorder characterized by severe osteoporosis, acroosteolysis of the distal phalanges, renal cysts, and other abnormalities.\textsuperscript{11,12} All reported variants associated with HCS are truncations that cluster in the last exon (exon 34) of NOTCH2.\textsuperscript{8,12-14}

The \textit{ATP8B1} gene encodes a type-4 P-typease which plays a role in bile formation and maintenance of lipid balance. Pathogenic variants in \textit{ATP8B1} are associated with various forms of hereditary cholestasis including autosomal dominant intrahepatic cholestasis pregnancy-1 (ICP1), autosomal recessive benign recurrent intrahepatic cholestasis-1 (BRIC1) and progressive familial intrahepatic cholestasis-1 (PFIC1).\textsuperscript{15} While the typical PFIC1 presentation is characterized by progressive cholestasis without extrahepatic findings observed in ALGS, patients from two families have been reported to have atypical PFIC1, which presented with cholestasis and clinical features consistent with ALGS (ALGS-like presentation). Those individuals tested negative for variants in the JAG1 and NOTCH2 genes, but carried biallelic loss-of-function variants in \textit{ATP8B1}.\textsuperscript{16,17}

**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. For the NOTCH2 gene, sequencing and deletion/duplication testing is not performed for exons 1-4.

Test Sensitivity

The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient’s clinical phenotype and family history. In general, the sensitivity is highest for individuals with clearly defined ALGS or a family history of the disease. Specific information about the sensitivity for each gene in selected populations is summarized in the table below.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Disease Association</th>
<th>Sensitivity</th>
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</thead>
<tbody>
<tr>
<td>ATP8B1</td>
<td>ATPase phospholipid transporting 8B1</td>
<td>AD/AR</td>
<td>ICP1 / BRIC1 / PFIC1</td>
<td>~41% of individuals with BRIC1 and ~30% of individuals with PFIC1&lt;sup&gt;18&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rare cause of atypical PFIC1&lt;sup&gt;16,17&lt;/sup&gt;</td>
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<tr>
<td>JAG1</td>
<td>Jagged canonical Notch ligand 1</td>
<td>AD</td>
<td>ALGS</td>
<td>~94% of individuals with ALGS&lt;sup&gt;19,20&lt;/sup&gt;</td>
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<tr>
<td>NOTCH2</td>
<td>Notch receptor 2</td>
<td>AD</td>
<td>ALGS / HCS</td>
<td>~1-2% of individuals with ALGS&lt;sup&gt;5,10&lt;/sup&gt;</td>
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<td>~83-100% of individuals with HCS&lt;sup&gt;11-14&lt;/sup&gt;</td>
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</table>

AD – Autosomal Dominant; ALGS – Alagille Syndrome; AR – Autosomal Recesive; BRIC1 – Benign Recurrent Intrahepatic Cholestasis-1; HCS – Hadju-Cheney syndrome; ICP1 – Intrahepatic Cholestasis of Pregnancy-1; PFIC1 – Progressive Familiar Intrahepatic Cholestasis-1

References: