

## Alport Syndrome Panel

### Disorder also known as:

Hematuria-nephropathy-deafness syndrome

### Panel Gene List:

CD151, COL4A3, COL4A4, COL4A5, COL4A6 and MYH9

### Clinical Features:

Alport syndrome (AS) is characterized by progressive renal disease, sensorineural hearing loss and ocular features.<sup>1</sup> Renal manifestations include hematuria, proteinuria, ultrastructural changes of the glomerular basement membrane and ultimately progression to end-stage renal disease (ESRD).<sup>2</sup> Hearing loss associated with AS tends to be mild to moderate, and may be more severe in higher frequencies.<sup>1</sup> Ocular symptoms are common and can include anterior lenticonus, central and peripheral fleck retinopathies, temporal retinal thinning and recurrent corneal erosions.<sup>3</sup>

AS is caused by pathogenic variants in the COL4A3, COL4A4 and COL4A5 genes, whereas an Alport-like presentation may be seen in individuals with pathogenic variants in the CD151 and MYH9 genes. Genotype-phenotype correlations for AS involving the onset of symptoms and severity of disease have been established. Males with COL4A5 X-linked AS tend to experience ESRD in their twenties or thirties, but the onset can range from late childhood to forties or later. Hearing loss tends to occur in late childhood or adolescence in males.<sup>1,4</sup> Symptoms in females with heterozygous pathogenic COL4A5 variants are variable, but tend to be milder and later in onset than in males. About 90% of female COL4A5 carriers have microscopic hematuria; however, some females remain completely asymptomatic. ESRD and hearing loss have been seen in 30-40% and 20% of adult females, respectively.<sup>1,5</sup> Submicroscopic deletions involving the 5' end of the COL4A5 gene, which typically extend into the 5' end of the adjacent COL4A6 gene, have been reported in association with Alport syndrome with diffuse leiomyomatosis (AS-DL).<sup>6,7</sup> AS-DL presents with the typical AS features as well as a benign overgrowth of smooth muscle cells, called leiomyomas. The leiomyomas associated with AS-DL commonly occur in the gastrointestinal, respiratory and female reproductive tracts.<sup>6,7</sup>

Individuals with autosomal recessive AS caused by pathogenic variants in the COL4A3 and COL4A4 genes tend to have symptoms similar in severity as males with X-linked AS, with ESRD typically occurring in the early twenties. Hearing loss can be seen in approximately 40-60% of individuals with autosomal recessive AS, with onset typically in the twenties or thirties.<sup>8,9</sup> Individuals with autosomal recessive pathogenic variants in the CD151 gene present with similarities to AS including nephropathy with proteinuria and deafness, with the addition of pretibial epidermolysis bullosa.<sup>10,11</sup>

Individuals with heterozygous COL4A3 and COL4A4 variants may only have hematuria or thin basement membrane defects and tend to have a milder phenotype as compared to individuals with autosomal recessive or males with X-linked AS, including a later onset and slower progression of renal disease and a lower incidence of extra renal symptoms.<sup>1,2,12</sup> Individuals with autosomal dominant Alport-like syndrome caused by pathogenic variants in the MYH9 gene present with macrothrombocytopenia (large platelets at low counts in the bloodstream) at birth and have a mild bleeding tendency. Some individuals also develop deafness, cataracts, and kidney dysfunction (which initially manifests as glomerular nephropathy) with a wide range of onset from infancy to adult life.<sup>13-15</sup>

### Genetics:

Approximately 80% of AS is inherited as X-linked trait; the remaining 20% is inherited as either an autosomal recessive or autosomal dominant. In rare cases, digenic inheritance has been described in individuals with heterozygous pathogenic variants in both the COL4A3 and COL4A4 genes.<sup>13</sup>

Multiple types of variants have been reported in COL4A3, COL4A4 and COL4A5.<sup>16-18</sup> Missense variants tend to be associated with a later age of onset of renal disease and hearing loss than loss-of-function variants in males with X-linked AS and in individuals with autosomal recessive AS.<sup>4,8</sup> Duplications within the COL4A5 gene have been reported in rare cases and as a founder variant within the French Polynesian population.<sup>19,20</sup>

## Test Methods:

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the 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 within adequate 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. For the CD151 gene, sequencing but not deletion/duplication analysis is performed.

## Clinical Sensitivity:

AS is a genetically heterogeneous disorder and there are many overlapping characteristics. 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. Additional information about the general clinical sensitivity of each gene is included in the table below.

Gene	Protein	Inheritance	Associated Phenotype	Sensitivity
CD151	CD151 Antigen	AR	Nephropathy with pretibial epidermolysis bullosa and deafness	Rare <sup>10,11</sup>
COL4A3	Collagen, type IV, alpha-3	AD / AR	AS	~12-15% of AS <sup>21-23</sup>
COL4A4	Collagen, type IV, alpha-4	AD / AR	AS	~5-8% of AS <sup>21-23</sup>
COL4A5	Collagen, type IV, alpha-5	XL	AS / AS-DL / AMME	~80-85% of AS <sup>21-23</sup>
COL4A6	Collagen, type IV, alpha-6	XL	AS-DL	5/6 individuals with ASDL with hetero- or hemizygous deletions

				involving COL4A5 and COL4A6 <sup>7</sup>
MYH9	Myosin, heavy chain 9, nonmuscle	AD	Alport-like syndrome with macrothrombocytopenia	20/27 individuals with an MYH9-related disorder <sup>15</sup>

AD = Autosomal Dominant; AR = Autosomal Recessive; XL = X-linked; AS = Alport syndrome; AS-DL = Alport syndrome with Diffuse Leiomyomatosis; AMME = Alport syndrome, Mental retardation, Midface hypoplasia and Elliptocytosis

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