Alport Syndrome Panel, Sequencing and Deletion/Duplication

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Alport syndrome (AS) is a spectrum of disorders that may range from isolated nonprogressive microscopic hematuria and proteinuria to progressive renal insufficiency, end stage renal disease (ESRD), eye findings, and sensorineural hearing loss (SNHL). The three genes causative for AS (*COL4A3*, *COL4A4*, and *COL4A5*) are critical to the collagen IV a345 network of basement membranes. Pathogenic variants in *COL4A5*, causative for approximately 80-85% of AS, are inherited in an X-linked (XL) manner. Approximately 15-20% of AS is autosomal dominant (AD) or autosomal recessive (AR) due to pathogenic variants in the *COL4A3* or *COL4A4* genes.

Disease Overview

Symptoms

Symptoms of AS and Related Disorders

Symptom Categories	X-Linked and Autosomal Recessive AS	Autosomal Dominant AS	*MYH9*-Related Disease
Renal symptoms	Renal disease progressing from microhematuria and proteinuria to renal insufficiency and ESRD • Males with X-linked AS: 60% have ESRD by age 30 and 90% by age 40 • Females with X-linked AS: 12% have ESRD by age 40, 30% by age 60, and 40% by age 80 • Individuals with autosomal recessive AS: most develop ESRD by age 30	Slowly progressive renal insufficiency presenting later in life	Early adult onset of renal disease, initially presenting as glomerular nephropathy
Hearing loss	Progressive SNHL in late childhood	Slowly progressive SNHL later in life	SNHL
Ocular issues	Anterior lenticonus • Presents in second or third decade of life • Observed in ~13% of males with X-linked AS • Associated with certain COL4A5 variants Maculopathy • Defined by whitish yellow flecks in the perimacular region • Observed in 14% of males with X-linked AS	Ocular lesions are uncommon	Cataracts

Featured ARUP Testing

Alport Syndrome Panel, Sequencing and Deletion/Duplication 3002685

Method: Massively Parallel Sequencing

- Recommended test to confirm carrier status or a diagnosis of AS or *MYH9*-related disease.
- Regions of low coverage and reported variants are confirmed by Sanger sequencing as necessary.

If a familial sequence variant has been previously identified, targeted sequencing for that variant may be appropriate; refer to the Laboratory Test Directory for additional information.

Symptom Categories	X-Linked and Autosomal Recessive AS	Autosomal Dominant AS	*MYH9*-Related Disease
	Corneal endothelial vesicles or corneal erosion in X-linked AS Posterior subcapsular cataracts in X-linked AS		
Other	Diffuse leiomyomatosis (benign smooth muscle cell proliferation) of the esophagus and tracheobronchial tree may occur in X-linked AS Thoracic and abdominal aortic aneurysms in a few males with X-linked AS at <40 years	–	Congenital presentation of large platelets and thrombocytopenia Adult onset of elevated liver enzymes

Etiology of Alport Syndrome

AS is caused by pathogenic variants in collagen genes that contribute to the collagen IV network of basement membranes.

Penetrance of Alport Syndrome and *MYH9*-Related Disease

- Complete for males with X-linked AS and individuals with AR *COL4A3* and *COL4A4* variants and *MYH9*-related disease
- Possible incomplete penetrance for AD *COL4A3* and *COL4A4* variants

Prevalence of Alport Syndrome

- 1/50,000 births[1]
- 0.2% of U.S. adults and 3% of children with ESRD have AS[2]

Inheritance

- *COL4A5*: XL
- *COL4A3* and *COL4A4*: AD and AR, depending on the variant
- *MYH9*: AD

Genotype-Phenotype Correlation

- Large *COL4A5* rearrangements, nonsense, frameshift, and splice site variants are associated with a 50% risk for ESRD by age 20, 90% risk of ESRD by age 30, and a 50% risk for SNHL by age 10.
- Pathogenic missense *COL4A5* variants confer a 50% risk for ESRD by age 30 and 50% risk of SNHL by age 20.
- Leiomyomatosis only occurs in individuals with a deletion of both *COL4A5* and *COL4A6* when the *COL4A6* breakpoint is in the second intron of the gene.

Test Interpretation

Methodology

This test is performed using the following sequence of steps:

- Selected genomic regions, primarily coding exons and exon-intron boundaries, from the targeted genes are isolated from extracted genomic DNA using a probe-based hybrid capture enrichment workflow.
- Enriched DNA is sequenced by massively parallel sequencing (MPS; also known as next generation sequencing [NGS]) followed by paired-end read alignment and variant calling using a custom bioinformatics pipeline. The pipeline includes an algorithm for detection of large (single exon-level or larger) deletions and duplications.
- Sanger sequencing is performed as necessary to fill in regions of low coverage and in certain situations, to confirm variant calls.
- Large deletion/duplication calls made using MPS are confirmed by an orthogonal exon-level microarray when sample quality and technical conditions allow.

Clinical Sensitivity

- Approaching 100% for AS[3-11]
- At least 98% for *MYH9*-related disease[12]

Analytic Sensitivity

For massively parallel sequencing:

Variant Class	Analytic Sensitivity (PPA) Estimate[a] (%) and 95% Credibility Region	Analytic Specificity (NPA)
SNVs	>99 (96.9-99.4)	>99.9
Deletions 1-10 bp[b]	93.8 (84.3-98.2)	>99.9
Insertions 1-10 bp[b]	94.8 (86.8-98.5)	>99.9
Exon-level[c] deletions	97.8 (90.3-99.8) [2 exons or larger] 62.5 (38.3-82.6) [single exon]	>99.9
Exon-level[c] duplications	83.3 (56.4-96.4) [3 exons or larger]	>99.9

[a]Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived. These values do not apply to testing performed by multiplex ligation-dependent probe amplification (MLPA).

[b]Variants greater than 10 bp may be detected, but the analytical sensitivity may be reduced.

[c]In most cases, a single exon deletion or duplication is less than 450 bp and 3 exons span a genomic region larger than 700 bp.

bp, base pairs; NPA, negative percent agreement; PPA, positive percent agreement; SNVs, single nucleotide variants

Limitations

- A negative result does not exclude a diagnosis of AS or *MYH9*-related disease.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - Variants outside the coding regions and intron-exon boundaries of targeted genes
  - Regulatory region and deep intronic variants
  - Breakpoints of large deletions/duplications
- The following may not be detected:
  - Deletions/duplications/insertions of any size by MPS
  - Noncoding transcripts
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants

Genes Tested

Gene Symbol	MIM #	Disorders	Inheritance
COL4A3	120070	Alport syndrome 2 Alport syndrome 3 Hematuria, benign familial	AR/AD
COL4A4	120131	Alport syndrome 2 Hematuria, benign familial	AR/AD
COL4A5	303630	Alport syndrome 1, X-linked	XL
MYH9	160775	Deafness 17 Macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss	AD

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