

Alport Syndrome: Clinical Spectrum and Therapeutic Advances



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Alport syndrome is a hereditary disorder characterized by kidney disease, ocular abnormalities, and sensorineural hearing loss. Work in understanding the cause of Alport syndrome and the molecular composition of the glomerular basement membrane ultimately led to the identification of *COL4A3*, *COL4A4* (both on chromosome 2q36), and *COL4A5* (chromosome Xq22), encoding the $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains of type IV collagen, as the responsible genes. Subsequent studies suggested that autosomal recessive Alport syndrome and males with X-linked Alport syndrome have more severe disease, whereas autosomal dominant Alport syndrome and females with X-linked Alport syndrome have more variability. Variant type is also influential—protein-truncating variants in autosomal recessive Alport syndrome or males with X-linked Alport syndrome often present with severe symptoms, characterized by kidney failure, extrarenal manifestations, and lack of the $\alpha 3$ - $\alpha 4$ - $\alpha 5$ (IV) network. By contrast, mild-moderate forms from missense variants display $\alpha 3$ - $\alpha 4$ - $\alpha 5$ (IV) in the glomerular basement membrane and are associated with protracted kidney involvement without extrarenal manifestations. Regardless of type, therapeutic intervention for kidney involvement is focused on early initiation of angiotensin-converting enzyme inhibitors. There are several therapies under investigation including sodium/glucose cotransporter 2 inhibitors, aminoglycoside analogs, endothelin type A antagonists, lipid-modifying drugs, and hydroxychloroquine, although targeting the underlying defect through gene therapy remains in preclinical stages.

Complete author and article information provided before references.

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INTRODUCTION

In 1927, Dr Cecil Alport reported a family in which several members were experiencing hematuria and deafness, with higher mortality observed in males compared with females.¹ However, it would take decades to understand the cause of the disease now known as Alport syndrome. A crucial step came in 1966 when collagen was first identified in the glomerular basement membrane (GBM) of canine kidneys and subsequently named type IV in 1973.^{2,3} Research on Goodpasture syndrome, an autoimmune condition that targets the GBM, also helped shed insight into Alport syndrome pathophysiology. In searching for the autoantigen in Goodpasture syndrome, investigators observed that Goodpasture syndrome autoantibodies bound to normal kidney tissue but not to Alport kidney tissue, suggesting that the antigen was absent.^{2,4} Electron microscopy provided granular characterization of the GBM in Alport syndrome, characterized by thickening, splitting, and lamellation.⁴

In 1981, type IV collagen was digested from the basement membranes of tumors found in an Engelbreth-Holm-Swarm tumor mouse line.^{2,5} Two isoforms of collagen, the $\alpha 1$ (IV) and $\alpha 2$ (IV) chains, were identified and found to be a ubiquitous component of basement membranes.^{2,5-7} By 1987, *COL4A1* and *COL4A2* were sequenced by molecular cloning and their complete primary protein structures established in human and mouse.^{2,5,6,8-20} Eventually all 6 type IV collagen isoforms, $\alpha 1$ (IV) to $\alpha 6$ (IV) chains, were reported, cloned, and described to share similar structures including: a C-terminal non-collagenous (NC1) domain

of ~230 residues, a ~1,400 residue collagenous Gly-X-Y repeat that forms a triple-helix with 2 other chains, and an N-terminal '7S' domain.^{10,21-27} The glycine residue is frequently mutated in Alport syndrome.²⁸ Unlike the ubiquitous basement membrane $\alpha 1$ (IV) and $\alpha 2$ (IV) chains, the other isoforms were observed to have more limited tissue distribution.^{3,29-33}

Thus, based on the observations of GBM abnormalities in Alport syndrome and the knowledge that type IV collagen comprised a major component of basement membranes, these then served as candidate genes. Linkage analysis suggested that X-linked forms of Alport syndrome were the most common, and subsequent studies evaluating different Alport syndrome kindreds identified mutations in *COL4A5*, residing on chromosome Xq22.^{25,26,34,35} Still, some families displayed autosomal recessive rather than X-linked inheritance patterns. *COL4A3* and *COL4A4*, located on chromosome 2q36, became of interest because of their expression in the GBM. In an initial discovery paper, homozygous mutations in both genes were reported in 4 consanguineous families.^{25,30,32,34}

GENETICS

By now, Alport syndrome is a well-known monogenetic disorder, resulting from pathogenic variants in *COL4A3*, *COL4A4*, or *COL4A5*. These genes encode the α (IV) chains in the mature GBM and basement membranes in the cochlea and base of the ocular lens, explaining the multi-system organ involvement seen in disease.³³ As indicated, there are 6 type IV collagen isoforms, encoded by different

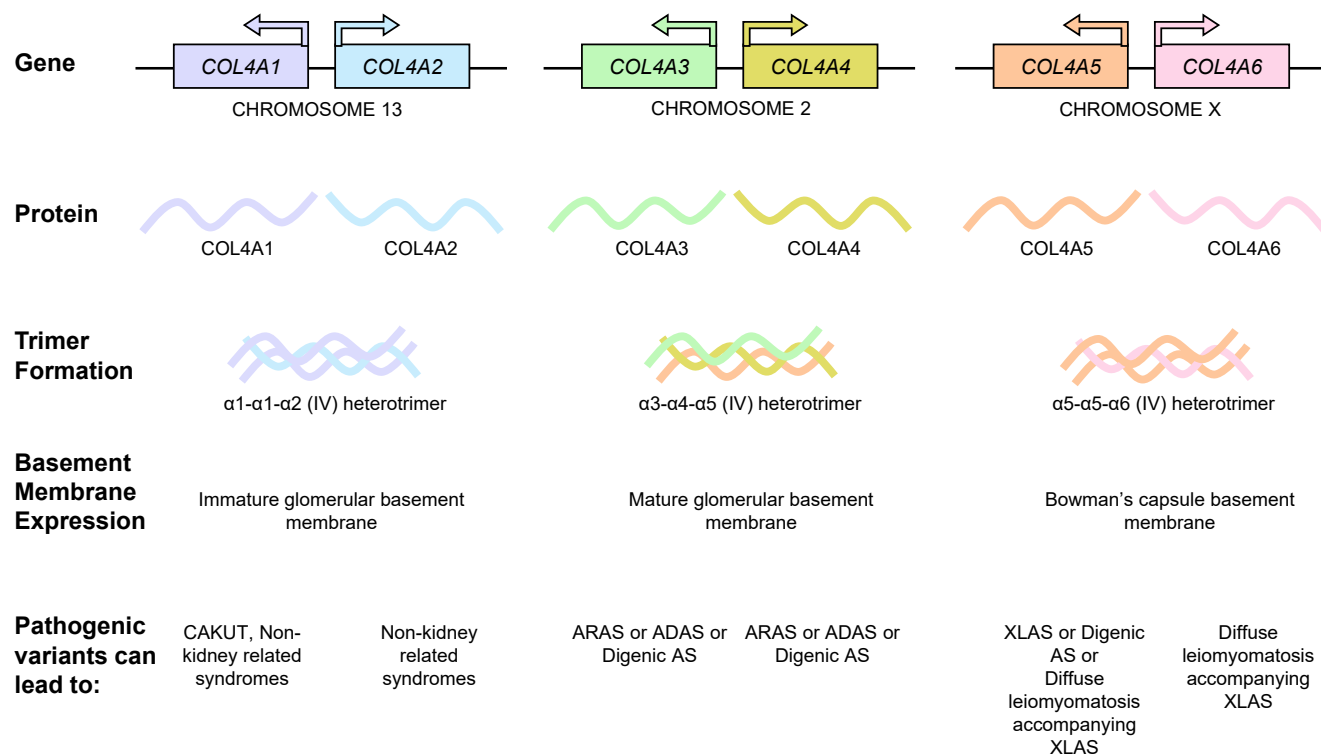


Figure 1. Type IV collagen from gene to protein trimerization to disease. The 6 collagens are arranged in a head-to-head configuration, with *COL4A1* and *COL4A2* on chromosome 13, *COL4A3* and *COL4A4* on chromosome 2, and *COL4A5* and *COL4A6* on chromosome X. The arrow above each gene indicates the direction in which transcription takes place. The collagen monomers come together to form heterotrimers, with the $\alpha 1\text{-}\alpha 1\text{-}\alpha 2$ (IV) expressed in the glomerular basement membrane during development, which later switches to the $\alpha 3\text{-}\alpha 4\text{-}\alpha 5$ (IV) in adulthood. Uniquely, the $\alpha 5\text{-}\alpha 5\text{-}\alpha 6$ (IV) heterotrimer is expressed in the basement membrane of Bowman's capsule. Pathogenic variants in these genes can lead to congenital abnormalities of the kidney and urinary tract (CAKUT) (*COL4A1*), autosomal recessive Alport syndrome (*COL4A3* or *COL4A4*), autosomal dominant Alport syndrome (*COL4A3* or *COL4A4*), digenic Alport syndrome (*COL4A3* and *COL4A4* or *COL4A3/COL4A4* and *COL4A5*), or diffuse leiomyomatosis accompanying X-linked Alport syndrome (*COL4A5* and *COL4A6*). ADAS, autosomal dominant Alport syndrome; ARAS, autosomal recessive Alport syndrome; AS, Alport syndrome; XLAS, X-linked Alport syndrome.

genes that are numerically labeled: *COL4A1*, *COL4A2*, *COL4A3*, *COL4A4*, *COL4A5*, and *COL4A6* (Fig 1).³⁶ Their encoded protein products assemble into heterotrimers, including $\alpha 1\text{-}\alpha 1\text{-}\alpha 2$ (IV), $\alpha 3\text{-}\alpha 4\text{-}\alpha 5$ (IV), and $\alpha 5\text{-}\alpha 5\text{-}\alpha 6$ (IV), to comprise network-forming collagen in basement membranes with restricted tissue expression (Fig 1). Within the GBM, type IV collagen is subjected to temporal regulation. The $\alpha 1\text{-}\alpha 1\text{-}\alpha 2$ (IV) heterotrimer is highly expressed during development with low expression of $\alpha 3\text{-}\alpha 4\text{-}\alpha 5$ (IV) (Fig 1).^{36,37} At the capillary loop stage of glomerular development, the $\alpha 1\text{-}\alpha 1\text{-}\alpha 2$ (IV) network is replaced by the $\alpha 3\text{-}\alpha 4\text{-}\alpha 5$ (IV) network.^{36,37} This developmental switching is postulated to be related to the biomechanical differences between the networks, with $\alpha 3\text{-}\alpha 4\text{-}\alpha 5$ (IV) conferring greater structural integrity to counter intracapillary flow.^{36,37}

The prevalence of Alport syndrome is unknown although is frequently reported as ranging from 1 in 5000 to 1 in 50,000 in the United States and Europe, respectively. These estimations, however, are based on clinical experience with imprecise inclusion criteria rather than epidemiologic studies.³⁸⁻⁴² Reports using the UK Biobank,

a genetically characterized population-based cohort of ~500,000 individuals, demonstrate an association of variants in *COL4A3* and *COL4A4* with Alport syndrome traits but only included adults between the ages of 40 to 69 at the time of recruitment.⁴³ By contrast, deCODE is a genetically characterized cohort of nearly the entire Icelandic population. In one deCODE study, 1 in 600 individuals in the Icelandic population are reported to carry a 2.5 kb *COL4A3* coding deletion (Gly289_Lys330del) or a *COL4A3* missense variant (rs200287952[A], p.Gly695Arg).^{44,45} Both variants are associated with hematuria (odds ratio [OR], 11.78; 95% confidence interval [CI], 7.26-19.11 and OR, 5.46; 95% CI, 2.94-10.16, respectively) while the former is also associated with proteinuria (OR, 2.17; 95% CI, 1.52-3.09). Neither variant was homozygous in any individual, and they were not identified in 4 documented Alport syndrome families in Iceland. In another study, rare missense variants predicted to be pathogenic by *in silico* programs involving the first Gly residue within the collagenous domain of *COL4A3*, *COL4A4*, and *COL4A5* were identified from gnomAD, a public database of 136,920 individuals with whole exome

or genome sequencing but without phenotypic data.²⁸ The bioinformatically predicted pathogenic missense variants were then evaluated in 39,421 individuals from the 100,000 Genomes Project, where 5.6% had a history of hematuria. Thus, most individuals did not have hematuria, and most predicted pathogenic variants were found in individuals without hematuria. In our own reported experience, we find that *in silico* programs tend to overestimate pathogenicity when compared to *in vitro* studies.⁴⁶ Categorization of single missense variants are vulnerable to misclassification for several reasons, which include not matching cases for population structure to controls and limited understanding of protein residue contribution to function. By comparison, the effect of protein-truncating variants is easier to understand and is among the highest levels of criteria for pathogenic classification by medical genetic society guidelines.⁴⁷

Alport syndrome follows 3 inheritance patterns: X-linked Alport syndrome, autosomal recessive Alport syndrome, and autosomal dominant Alport syndrome.³³ X-linked Alport syndrome is caused by pathogenic variants in *COL4A5* and in the past has been reported to be the most common form. Many papers report that X-linked Alport syndrome accounts for 80% of cases, but newer studies are challenging this statistic.^{28,33,48} Autosomal recessive Alport syndrome and autosomal dominant Alport syndrome are caused by pathogenic variants in *COL4A3* and/or *COL4A4*, respectively.^{33,48} Although autosomal forms were previously felt to account for a minority of cases, this is now changing in light of these more recent studies. Autosomal recessive Alport syndrome results from homozygous or compound heterozygous rare variants in *COL4A3* or *COL4A4*, whereas autosomal dominant Alport syndrome results from heterozygous rare variants in these genes.

Alport syndrome can also follow digenic inheritance, which is the presence of 2 pathogenic variants in different type IV collagen genes.⁴⁹ There have been several reports of digenic inheritance in Alport syndrome, which is another factor that likely contributes to variability in clinical presentation.⁴⁹⁻⁵¹

TERMINOLOGY

Like other kidney disorders, terminology is an issue that has been discussed in Alport syndrome for some time with varying perspectives. Previously, thin basement membrane nephropathy was a descriptive diagnosis used to describe this pathologic pattern, observed to be usually associated with mild clinical features of hematuria and some albuminuria with favorable kidney prognoses.⁵²⁻⁵⁵ However, as the experience with genetic testing expands, there is now recognition that previously labeled thin basement membrane nephropathy is often a manifestation of autosomal dominant disease, which has more variable course and can include progression albeit later in life with exceptions.^{50,56-60} Similarly, females heterozygous for pathogenic *COL4A5* variants have been previously labeled

as carriers, but we now recognize that these cases also have variability and can progress.^{61,62} As a result, a consensus statement made by one working group in Alport syndrome has recommended that the term carrier be abandoned with wording felt to be important so as not to deprive individuals from necessary monitoring and intervention by specialists.⁵⁶

These observations also suggest that Alport syndrome does not follow classic Mendelian patterns but is more consistent with additive inheritance, the model that is assumed in most genome-wide association studies. With additive inheritance, phenotype varies cumulatively with the number of pathogenic variants that are in trans involving the disease-associated genes. By contrast, in true autosomal dominant and autosomal recessive disease, the number of variants has no influence on phenotypic severity. So, for instance, a pathogenic dominant variant should manifest with the same phenotypic severity if present in 1 copy or 2 copies in trans. However, there is evidence that at least one protein-truncating variant, *COL4A4* (rs35138315, p.Ser969X, minor allele frequency = 6.7×10^{-4}), follows an additive inheritance pattern, which has been identified as strongly associated in the UK Biobank with hematuria and as a common cause of autosomal recessive Alport syndrome.^{45,63-66} In UK Biobank studies, this variant is observed mostly as heterozygous in participants with hematuria, where cases have a mean age of 60 years, urinary albumin-creatinine ratio (UACR) of 1.19 mg/mmol and estimated glomerular filtration rate (eGFR) of 88 mL/min/1.73 m².⁶⁷ This same variant, however, is described in reports of autosomal recessive Alport syndrome in which affected individuals manifest the traditional constellation of symptoms and extrarenal involvement.

The challenge thus has been what constitutes Alport syndrome? An all-encompassing term would be to define Alport syndrome as any condition in which a type IV collagen variant that is felt to be causative for the phenotypic traits of Alport syndrome is included, regardless of number of symptoms or organs with clinically significant involvement, as recommended by the Alport Syndrome Classification Working Group.⁵⁶ With this cautious approach, the risk of excluding patients from specialist clinical care will be potentially lower.⁴⁵

CLINICAL PRESENTATION

Alport syndrome includes kidney, auditory, and ocular involvement, with these 3 organs unified by the presence of $\alpha3\text{-}\alpha4\text{-}\alpha5$ (IV) in the basement membranes (Fig 2). The natural history of Alport syndrome has been best characterized in males with X-linked Alport syndrome.^{68,69} Two large family studies in Europe and North America identified a strong correlation between the variant type and disease severity (Fig 3).^{68,69} Variants with substantial effect on the encoded protein, such as large deletions/rearrangements, protein truncations, and frameshifts, are associated with more severe disease and are more likely to have extrarenal

Clinical Manifestations	Microscopic hematuria	Albuminuria with some macroscopic hematuria attacks possible; eye deformities and hearing loss may begin	Albuminuria and hematuria worsen; eGFR declines; eye deformities and hearing loss worsens	Kidney failure
Electron Microscopy Pathology	Some GBM thinning and splitting may be observed	GBM begins to thicken; splitting may worsen	GBM has a basketweave appearance; Some glomerulosclerosis and foot process effacement may be visible	Severe GBM abnormalities; extreme glomerulosclerosis and foot process effacement

Figure 2. Clinical characteristics and GBM features observed in Alport syndrome. There is wide clinical variability in Alport syndrome. Stages of progression for the most severe forms are shown. eGFR, estimated glomerular filtration rate; GBM, glomerular basement membrane.

involvement. By contrast, variants with more minor effects on the encoded protein, including missense and splice site variants, can attenuate the phenotype.

Affected X-linked Alport syndrome males, in which there is only 1 X chromosome, are more severely affected than heterozygous X-linked Alport syndrome females, in which there are 2. In heterozygous X-linked Alport syndrome females, there is large variability in clinical presentation as a result of X-linked inactivation, in which the maternal or paternal inherited X chromosome is marked for inactivation randomly in cells.⁷⁰ Male patients with X-linked Alport syndrome and patients with autosomal recessive Alport syndrome share similar clinical courses, whereas patients with autosomal dominant Alport syndrome display large clinical variability.

Kidney Involvement

Kidney involvement includes glomerular hematuria, albuminuria, GBM abnormalities, and kidney failure.

Hematuria is often the first sign and can occur as early as infancy. Males with X-linked Alport syndrome and affected individuals with autosomal recessive Alport syndrome can present with hematuria in their first year of life.^{60,68,71,72} Albuminuria typically occurs later, and depending on the duration and degree, decreased eGFR subsequently follows. The age at which kidney failures occurs, if it is to happen, has broad variability.^{68,69,73} In one retrospective study, the median ages at kidney failure for X-linked Alport syndrome males with large deletion/protein-truncating, splice site, and missense variants were 25 (95% CI, 21-31), 28 (95% CI, 26-32), and 37 (95% CI, 34-40) years, respectively ($P < 0.001$ for the former 2 versus the latter), indicating allelic heterogeneity.⁶⁹ In another study, 5 heterozygous X-linked Alport syndrome females had a progressive course that varied from 12 to 83 years at the time of kidney failure.⁷⁴ Jais et al⁶¹ observed that 12% of heterozygous X-linked Alport syndrome females reached kidney failure by age 40 and 30%-40%

$\alpha3$-$\alpha4$-$\alpha5$ (IV) Expression	Some expression		No expression
Variant	Missense	Splice site	High impact: protein truncating, nonsense, frameshift, deletion, rearrangement
AS Subtype	ADAS Females with XLAS		ARAS Males with XLAS

Figure 3. Factors that affect disease severity. The severity of Alport syndrome is influenced by the type of pathogenic variant(s) in COL4A3/COL4A4/COL4A5 and pattern of inheritance, which in turn affects the levels of $\alpha3$ - $\alpha4$ - $\alpha5$ (IV) expression in the glomerular basement membrane. The combination of these factors contributes to the broad spectrum of disease observed clinically. ADAS, autosomal dominant Alport syndrome; ARAS, autosomal recessive Alport syndrome; AS, Alport syndrome; CAKUT, congenital abnormalities of the kidney and urinary tract; XLAS, X-linked Alport syndrome.

by age 60 in their cohort of 195 X-linked Alport syndrome families. In the same study, 70% of hemizygous men at the age of 30 and 90% at age 40 had reached kidney failure.

Kidney Pathology and Imaging the GBM

Electron microscopy is a useful tool in imaging the GBM (Fig 2). In the earlier stage, GBM thinning and splitting are observed.⁷⁵ As the disease progresses, the GBM becomes thickened with more pronounced splitting.^{75,76} GBM thickening can follow the classic basket-weave pattern, where short fragments of fibrils are oriented perpendicular to the GBM. These advanced pathologic features are accompanied by albuminuria and reduced eGFR.⁷⁵⁻⁷⁷ In the final stages, Alport syndrome is associated with focal and segmental glomerulosclerosis (FSGS), an accumulation of extracellular matrix proteins in glomeruli along with podocyte foot process effacement.^{75,78,79} Some cases of Alport syndrome present as adult-onset FSGS without obvious extrarenal symptoms and therefore elude accurate diagnosis.^{65,66,80} This clinical scenario thus highlights the importance of electron microscopy for diagnosis, in particular its usefulness if genetic testing is not available or is inconclusive.

Staining for Type IV Collagen

Staining for type IV collagen is not typically done in routine clinical kidney biopsy testing but can be informative when performed. In studies, large deletions/rearrangements and protein-truncating (ie, nonsense or frameshift) variants in males with X-linked Alport syndrome or patients with autosomal recessive Alport syndrome are associated with loss of GBM expression of $\alpha3\text{-}\alpha4\text{-}\alpha5$ (IV) and earlier progression to kidney failure, sensorineural deafness, and ocular lesions compared with missense or splice site mutations (Fig 3).^{68,69} In comparison, $\alpha3\text{-}\alpha4\text{-}\alpha5$ (IV) will be synthesized in autosomal dominant Alport syndrome and females with X-linked Alport syndrome. It will also be expressed in X-linked Alport syndrome males and autosomal recessive Alport syndrome due to missense and splice site mutations. Thus, the absence of $\alpha3\text{-}\alpha4\text{-}\alpha5$ is informative of the underlying genetics and can be prognostic.

Ocular abnormalities and sensorineural hearing loss

Ocular abnormalities are common due to the presence of type IV collagen in the lens capsule, Descemet's membrane, Bowman's membrane, and retinal pigment epithelium of the eye.^{81,82} Most ocular abnormalities occur as the disease progresses, with some associated with kidney failure before the age of 30 years in males with X-linked Alport syndrome.^{82,83} Characteristic ocular abnormalities include corneal opacities, anterior lenticonus, cataract, and retinal abnormalities.⁸²

Ocular examination can assist in diagnosing Alport syndrome, although the absence of findings does not rule

out disease. In a study of 14 families with X-linked Alport syndrome, 6 of 15 affected males had ocular abnormalities including lenticonus (1) or retinopathy (5).⁸⁴ Despite this, no eye findings were described in heterozygous COL4A5 females.⁸⁴

Like ocular abnormalities, sensorineural hearing loss typically develops and worsens as the disease progresses. Approximately 12% of female patients with X-linked Alport syndrome and 90% of male patients with X-linked Alport syndrome present with hearing loss by 40 years old, which contrasts with autosomal dominant Alport syndrome, where deafness is less commonly reported.³³ The $\alpha3\text{-}\alpha4\text{-}\alpha5$ (IV) network comprises the basilar membrane in the cochlea. Tension fibroblasts generate tension on the basilar membrane for high frequency sound detection, and the absence of $\alpha3\text{-}\alpha4\text{-}\alpha5$ (IV) by staining, which is only possible to do after death, is associated with sensorineural hearing loss.⁸⁵

THERAPEUTICS FOR KIDNEY DISEASE IN ALPORT SYNDROME

Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs) are the mainstay of treatment in Alport syndrome. In the past few years, there has been renewed interest in developing drugs for chronic kidney diseases including Alport syndrome. Several investigational drugs are at different stages of development including sodium/glucose cotransporter 2 inhibitors (SGLT2is), aminoglycoside analogs, endothelin type A antagonists, lipid-modifying drugs, hydroxychloroquine (HCQ), anti-miR-21, bardoxolone methyl, and gene replacement therapy. Agents in phase 2 clinical trials are shown in Table 1.

Angiotensin-converting Enzyme Inhibitors and ARBs

Angiotensin-converting enzyme inhibitors were initially shown to reduce proteinuria and blood pressure in a small study of 3 patients with a clinical diagnosis of Alport syndrome in 1996.⁸⁶ Subsequently, a study in a Col4a3 knockout mouse model demonstrated that treatment with ramipril, initiated before the onset of proteinuria, doubled the life expectancy of Alport syndrome-affected mice.⁸⁷ In 2012, results from a noninterventional observational study involving 283 Alport syndrome patients followed for over 2 decades showed that initiation of angiotensin-converting enzyme inhibitors significantly delayed age of kidney failure.⁸⁸ There was a statistically significant difference in the delay of kidney replacement therapy between the nontreated and each of the 3 treatment groups anywhere between 3 to 40 years ($P < 0.001$ for each comparison), with the greatest delay observed in the hematuria or microalbuminuria (treatment) group.⁸⁸

However, to answer the question of whether early angiotensin-converting enzyme inhibitor/ARB initiation at

Table 1. Current Phase 2 Clinical Trials Testing Safety and Efficacy of Different Agents in Alport Syndrome.

Agent	Class	Sponsor	Study Population	Clinical Trials Link
ELX-02	Aminoglycoside analog	Eloxx Pharmaceuticals	Alport syndrome (nonsense mutations)	https://clinicaltrials.gov/ct2/show/NCT05448755
Atrasentan	Endothelin A receptor antagonist	Chinook Therapeutics U.S., Inc	IgA nephropathy FSGS Alport syndrome Diabetic kidney disease	https://clinicaltrials.gov/ct2/show/NCT04573920
Sparsentan	Dual endothelin Angiotensin Receptor Antagonist	Travere Therapeutics	FSGS Minimal change IgA nephropathy IgA vasculitis Alport syndrome	https://clinicaltrials.gov/ct2/show/NCT05003986
R3R01	Lipid-modifying	River 3 Renal Corp	Alport syndrome FSGS	https://clinicaltrials.gov/ct2/show/NCT05267262
Hydroxychloroquine sulfate (HCO) Benazepril hydrochloride	HCO ACEi	Shanghai Children's Hospital	X-linked Alport syndrome	https://clinicaltrials.gov/ct2/show/NCT04937907

ACEi, angiotensin-converting enzyme inhibitor; FSGS, focal and segmental glomerulosclerosis; HCO, hydroxychloroquine.

the time of isolated hematuria and/or microalbuminuria in children and youth is effective, a randomized control study known as EARLY PROTECT was designed to test ramipril in Alport syndrome individuals with an eGFR above 90 mL/min/1.73 m² who were between the ages of 24 months to 18 years old.⁸⁹ Of 66 patients, 43 were part of an open-label treatment arm due to pretreatment with angiotensin-converting enzyme inhibitors or lack of parental consent; 10 were assigned to the placebo group, and 12 were assigned to ramipril over 2 months.⁸⁹ There were no safety issues (adverse event risk ratio, 1.00; 95% CI, 0.66-1.53; *P* = 0.9), and ramipril reduced the slope of albuminuria and decrease in eGFR between treated and untreated groups by almost 50%, although it was not statistically significant (hazard ratio, 0.51; 95% CI, 0.12-2.20; *P* = 0.4).⁸⁹

SGLT2is

The use of SGLT2is for treatment of diabetic kidney disease has become popularized in recent years due several large, randomized control studies demonstrating benefit and has been approved for this indication by several federal agencies including the Food and Drug Administration and Health Canada.⁹⁰⁻⁹⁵ This in turn has bolstered enthusiasm for studying SGLT2is in nondiabetic proteinuric kidney diseases. The purported mechanism of action is SGLT2i-mediated afferent arteriole vasoconstriction.⁹⁰

Recently the EMPA-KIDNEY trial with 6,609 chronic kidney disease patients enrolled to assess the efficacy of empagliflozin was reported.⁹⁶ The mean age of the participants was 63.8 years, with mean (\pm standard deviation) eGFR 37.3 \pm 14.5 mL/min/1.73 m² and a median UACR of 329. Approximately 26% of patients were diagnosed with glomerular disease.⁹⁶ The study's primary outcome was the composite of differences in the progression of kidney disease or death from cardiovascular causes. After a

median follow-up time of 2.0 years, there was a statistically significant difference in the primary outcome in the empagliflozin compared with the placebo group (hazard ratio, 0.72; 95% CI, 0.64-0.82; *P* < 0.001).⁹⁶ The geometric mean UACR ratio was 19% lower in the treated compared with placebo group (95% CI, 15-30). There was no increase in serious adverse events in the empagliflozin group.⁹⁶

In an observational, prospective case series assessing the effects of SGLT2is on early kidney function and safety, 6 patients diagnosed with Alport syndrome and/or FSGS via kidney biopsy, genetic testing, or both were treated with SGLT2is along with their usual medications, although one patient was forced to stop angiotensin-converting enzyme inhibitors due to angioedema.⁹⁰ SGLT2is were given once daily, with the mean duration of treatment 4.5 months.⁹⁰ There was a 40% decline in the mean UACR from 1,827 (\pm 1560) mg/g to 1,127 (\pm 854) mg/g and no differences in median eGFRs before and after treatment.⁹⁰

Larger trials with greater Alport syndrome patient enrollment are needed to determine if SGLTis will have benefit in these individuals clinically, although its use is becoming increasingly popular while waiting for more robust data. However, this will take time, and many do not want to deprive patients of this therapy in the meantime.

Aminoglycoside Analogs

Aminoglycosides have been used in genetic diseases including cystic fibrosis and Duchenne muscular dystrophy by virtue of their action to correct nonsense variants.^{97,98} This is achieved by the therapeutic binding to the 16S rRNA amino-acyl site, which results in the read-through of the stop codon, preventing premature peptide termination.⁹⁷⁻¹⁰¹

In a phase 1 randomized, double-blind study, ELX-02, an aminoglycoside analog, was tested to assess its safety

and pharmacokinetics in 62 healthy participants over 29 days.¹⁰¹ Ninety percent of participants experienced at least 1 treatment-emergent adverse event, with the most common being injection site reaction, and 7% experienced hearing impairment.¹⁰¹ ELX-02 is now in phase 2 clinical trials for patients with X-linked Alport syndrome and autosomal recessive Alport syndrome that are known to have nonsense variants.

Endothelin Type A Antagonists

Endothelin 1 (ET-1) produced by endothelial cells has also become a target of study as a result of its upregulation in Alport glomeruli through biomechanical strain-sensitive activation of mesangial actin dynamics.¹⁰²⁻¹⁰⁴ The administration of an endothelin A receptor antagonist to prevent ET-1 binding in an X-linked Alport syndrome mouse model over 5 weeks resulted in a reduction in proteinuria and serum urea nitrogen, the inhibition of mesangial filopodial invasion and the normalization of GBM morphology.¹⁰² Currently, there are 2 clinical studies evaluating endothelin receptor antagonists.

Lipid-modifying Drugs

Fatty acids are important energy sources for the kidney, especially tubular cells, whereas glomerular cells mostly prefer glucose as a first choice.¹⁰⁵ Cholesterol and sphingolipids also have a substantial role as the structural component of the podocyte slit diaphragm.¹⁰⁶ Deposition of fatty acids in the mitochondrial matrix leads to the production of reactive oxygen species, which, together with lipid peroxidation, results in damage to several organelles, particularly the mitochondria. Cell apoptosis is also triggered, leading to the release of proinflammatory/profibrotic mediators.^{107,108} The most susceptible renal cells to lipotoxicity are podocytes.¹⁰⁵

Several studies have shown a prevalence of lipid accumulation in mouse models of Alport syndrome and FSGS. In one study, hydroxypropyl- β -cyclodextrin, which is known to bind to cholesterol, was shown to be beneficial in a FSGS NFAT mouse model.¹⁰⁹ In another, hydroxypropyl- β -cyclodextrin administered for 4 weeks in Col4a3 Alport syndrome mice resulted in reduced kidney lipid accumulation, fibrosis, podocyte foot process effacement in the glomeruli and a decrease in UACR compared with littermate controls.¹⁰⁹ As such, various drugs have been developed to reduce the effects of lipotoxicity in kidney diseases, one being R3R01, a novel lipid-modifying agent currently in phase 2 clinical trials.

HCQ

HCQ is an antimalaria agent that is also used to treat certain autoimmune conditions, including lupus erythematosus and inflammatory arthritis.¹¹⁰ HCQ suppresses the activation of toll-like receptors on the surface of endosomes.¹¹⁰ Toll-like receptors are responsible for the activation of interferon-regulated genes and tumor necrosis factor α ,

which play an important role in inflammation pathways.¹¹⁰ HCQ has been evaluated in membranous lupus nephritis and IgA nephropathy. In a randomized controlled trial of 60 IgA nephropathy participants treated for 6 months with HCQ, there was a statistically significant reduction in proteinuria in the treated compared to placebo group (percentage change -48.4% vs 10.0% , $P < 0.001$; 0.9 g/d vs 1.9 g/d, $P = 0.002$) and without any reported serious adverse effects.¹¹¹ Clinical trials for HCQ are currently in phase 2 testing for individuals with X-linked Alport syndrome.¹¹²

Anti-miR-21

MicroRNA-21 (miR-21) expression has been found to drive fibrosis, and preclinical work in Col4a3 knockout mice supported use of oligonucleotides that inhibit its action.^{113,114} These data formed the rationale for the development of a phase 2 clinical trial (HERA) testing anti-miR-21 (lademirsén or SAR339375) in Alport syndrome. Some of the eligibility criteria included ages 18-55 years, with an eGFR between 35-90 mL/min/1.73 m² based on CKD-EPI (chronic kidney disease epidemiology collaboration) at the time of enrollment and an eGFR decline of ≥ 4 mL/min/1.73 m²/year within 3 years before the study (<https://clinicaltrials.gov/ct2/show/NCT02855268>). Disappointingly, the study was terminated due to the interim analysis for futility failing to demonstrate a meaningful improvement in the primary endpoint of annualized eGFR decline.

Bardoxolone Methyl

Bardoxolone methyl was another investigational drug that was tried in Alport syndrome. Bardoxolone methyl was thought to promote resolution of inflammation by activating nuclear factor erythroid 2-related factor 2 and inhibition of nuclear factor κ -light-chain enhancer of activated B cells.^{115,116} Clinical trials for bardoxolone methyl began in 2011 in patients with type 2 diabetes and stage 3 chronic kidney disease but was associated with excess risk of hospitalization or death from heart failure (relative risk, 1.82; 95% CI, 1.32-2.55; $P < 0.001$).¹¹⁷ The drug increased eGFR but also increased urinary albumin excretion, perhaps due to raised intraglomerular pressure, which would be predicted to lead to kidney function decline over time.¹¹⁸

In 2017, a clinical trial (CARDINAL; <https://clinicaltrials.gov/ct2/show/NCT03019185>) re-emerged involving Alport syndrome patients.^{116,117} The trial's phase 3 data was recently published reporting outcomes in 157 Alport syndrome participants. The mean age (standard deviation) was 39 (15) and 40 (16) years in the bardoxolone methyl and placebo groups, respectively. The mean baseline eGFR was 63 mL/min/1.73 m² for both with a geometric mean baseline UACR of 148 and 134 mg/g.¹¹⁹ The primary endpoint was a change from baseline eGFR after 48 and 100 weeks.^{116,119} The between-group

differences for the primary endpoint was statistically significant at both times, favoring bardoxolone methyl (48 weeks, 9.2; 97.5% CI, 5.1-13.4; $P < 0.001$ and 100 weeks, 7.4; 95% CI, 3.1-11.7; $P = 0.0008$).¹¹⁹ A post hoc analysis at week 104 using all available eGFR data did not reach statistical significance, however. Additionally, discontinuations from treatment were more frequent among patients randomized to bardoxolone methyl (10 of 77) with most due to protocol-specified criteria being met for increases in serum transaminases. Despite this, there were more serious adverse events reported in the placebo compared with the treatment group ($n = 15$ and 8).¹¹⁹ The FDA did not approve bardoxolone methyl, citing lack of evidence for efficacy and concerns over safety, particularly potential for hepatotoxicity.

Gene Replacement Therapy

The therapeutics discussed thus far focus on managing and slowing disease progression. Gene replacement therapies, however, focus on restoring gene function to produce functional proteins. In Alport syndrome, all development of gene therapies is in preclinical stages.

A creative gene editing strategy that has been tested is exon skipping therapy where antisense oligonucleotides bind to exonic splicing enhancer regions causing the splicing out of whole exons. In this approach, exons that harbor truncating variants are targeted and replaced with in-frame deletion mutations, lessening the disease phenotype.¹²⁰ In the only reported study, subcutaneous injections 1-2 times a week resulted in partial recovery of COL4A5 staining, reduced proteinuria, and delayed kidney failure in Alport mice with a nonsense mutation in *Col4a5*.¹²⁰⁻¹²²

In another study, podocyte specific expression of an inducible *Col4a3* transgene was applied to *Col4a3* knockout mice.¹²³ Normal GBM was observed in transgenic lines, even at 23 weeks of age, with little to no albuminuria compared with *Col4a3* knockout littermates, which reach kidney failure and death by 8 weeks.¹²³

Finally, CRISPR/Cas9 genome editing has been tested *in vitro* but is also in early stages of development. In one report, CRISPR/Cas9 gene editing was applied to podocytes cultured from Alport syndrome patient urine, and results indicated that <40% of pathogenic variants were corrected.¹²⁴ Additionally, undesired effects were observed, including insertions and deletions.¹²⁴

CONCLUSION

Alport syndrome is an inherited disorder caused by pathogenic variants in *COL4A3*, *COL4A4*, or *COL4A5*, encoding the $\alpha3$ - $\alpha4$ - $\alpha5$ (IV) chains expressed in the mature GBM of the kidney. Alport syndrome presents as a broad spectrum of disease influenced by genetic factors in affected individuals with clinical manifestations including hematuria, albuminuria, kidney failure, ocular abnormalities, and sensorineural hearing loss.

Angiotensin-converting enzyme inhibitors and ARBs are currently the mainstay of therapy, reducing albuminuria and slowing progression, which is the most marked with early intervention. Additionally, SGLT2is have promise and are already being used as adjunctive therapy to angiotensin-converting enzyme inhibitors/ARBs, while awaiting larger clinical trials in Alport syndrome and other proteinuric conditions. In recent years, there has been renewed interest in elaborating the therapeutic spectrum further, and several agents have been or are currently in clinical trials including aminoglycoside analogs, endothelin type A antagonists, lipid-modifying drugs, HCQ, anti-miR-21, bardoxolone methyl, and gene replacement therapy. These novel therapeutics highlight the growing knowledge in Alport syndrome and provide optimism for improved clinical outcomes in the future.

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