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Genetic technology in the targeted therapy of Alport Syndrome.

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ABSTRACT

Introduction and aim of the study: Alport syndrome is the most common inherited chronic kidney disease, with three distinct patterns of inheritance: X-linked, autosomal, and digenic. Currently, there is no curative treatment for Alport syndrome. This review aims to provide updated knowledge about Alport syndrome, including its clinical and genetic characteristics and therapies that slow disease progression.

Material and Methods: A systematic literature search was conducted using PubMed and Google Scholar, with the following keywords: "gene," "therapy," "Alport," and "syndrome."

Description of the state of knowledge: Gene therapy, which uses genetic material to prevent or treat diseases, offers promising prospects for Alport syndrome. The results of the metaanalysis suggest that gene editing by CRISPR/Cas9, exon-skipping using an antisense-oligonucleotide, premature termination codon readthrough, anti-miRNA-21 oligonucleotides, protein replacement, pharmacological chaperones and X-chromosome reactivation therapies are a feasible approach for some patients with Alport syndrome.

Summary: Targeting defective collagen chains early in the disease through gene therapy may have the greatest potential to reverse this disorder. Therefore, we strongly believe Alport syndrome will become a treatable condition in the near future using gene editing techniques.

Key words: Alport, syndrome, genetic, therapy, CRISPR/Cas9, exon-skipping, premature termination codon readthrough, anti-miRNA-21 oligonucleotides, protein replacement, pharmacological chaperones, X-chromosome

INTRODUCTION AND PURPOSE

Alport syndrome (AS) is the most common inherited chronic kidney disease (CKD), with three distinct patterns of inheritance: X-linked, autosomal, and digenic. AS is characterized by proteinuria and hematuria, which lead to chronic inflammation, fibrosis, and progressive kidney disease. These complications often culminate in end-stage renal failure (ESRF) early

in life. The disease accounts for 30–40% of proteinuric CKD cases in children and over 10% of end-stage kidney disease (ESKD) cases in young adults. For instance, children with classical forms of AS are at an extremely high risk of developing ESRF early, significantly reducing their quality of life and life expectancy. [1] Numerous patient testimonies emphasize the unmet medical needs in children with CKD. Resources such as the Alport Syndrome Foundation homepage, the FDA's "Voice of Patients" report, [2] and a video portrait by the German Ministry of Education and Research with English subtitles highlight these challenges. [3]

Currently, there is no curative treatment for AS. Existing therapeutic options, including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), aim to alleviate glomerular filtration pressure, reduce renal injury, and delay the progression of renal dysfunction. [4–6] For patients with ESRF, treatment is limited to dialysis or kidney transplantation. [1] However, the limited effectiveness of these interventions underscores the urgent need for novel therapeutic strategies to improve patient outcomes.

This review provides updated insights into the clinical and genetic characteristics of AS, along with an overview of current therapies that primarily slow disease progression. Gene therapy (GT), which uses genetic material to prevent or treat diseases, represents a promising approach for AS treatment. Additionally, this review emphasizes the importance of early diagnosis and proactive management to mitigate disease progression.

MATERIAL AND METHODS

A systematic literature search was conducted using PubMed and Google Scholar with the following search terms: "gene," "therapy," "Alport," and "syndrome." The search focused on articles published between 2020 and 2025. Additionally, frequently cited publications predating this period were included. The inclusion criteria encompassed observational studies, clinical trials, meta-analyses, and systematic reviews published in peer-reviewed journals. Non-English articles and book chapters were excluded from the analysis.

STATE OF THE ART DESCRIPTION

Background of Alport Syndrome

Alport syndrome is predominantly caused by mutations in three genes encoding type IV collagen alpha chains: COL4A3 (α3), COL4A4 (α4), and COL4A5 (α5). [7] These genes play a crucial role in the biosynthesis of type IV collagen, a key component of the glomerular basement membrane (GBM). Type IV collagen constitutes approximately 50% of the total protein mass of the GBM and is critical for its structural stability. The GBM, a thin extracellular matrix, functions as a selective barrier, preventing the passage of blood cells and proteins from the bloodstream into the urinary tract. Mutations affecting α-chains lead to dysfunctional GBM, which can result in clinical manifestations such as sensorineural deafness, ocular abnormalities, hematuria, proteinuria, and the progression to CKD. [8–10] XLAS, caused by defects in the COL4A5 gene, accounts for approximately 80% of AS cases. The remaining cases, categorized as autosomal or digenic, are associated with mutations in the COL4A3 or COL4A4 genes. [11] (Figure 1).

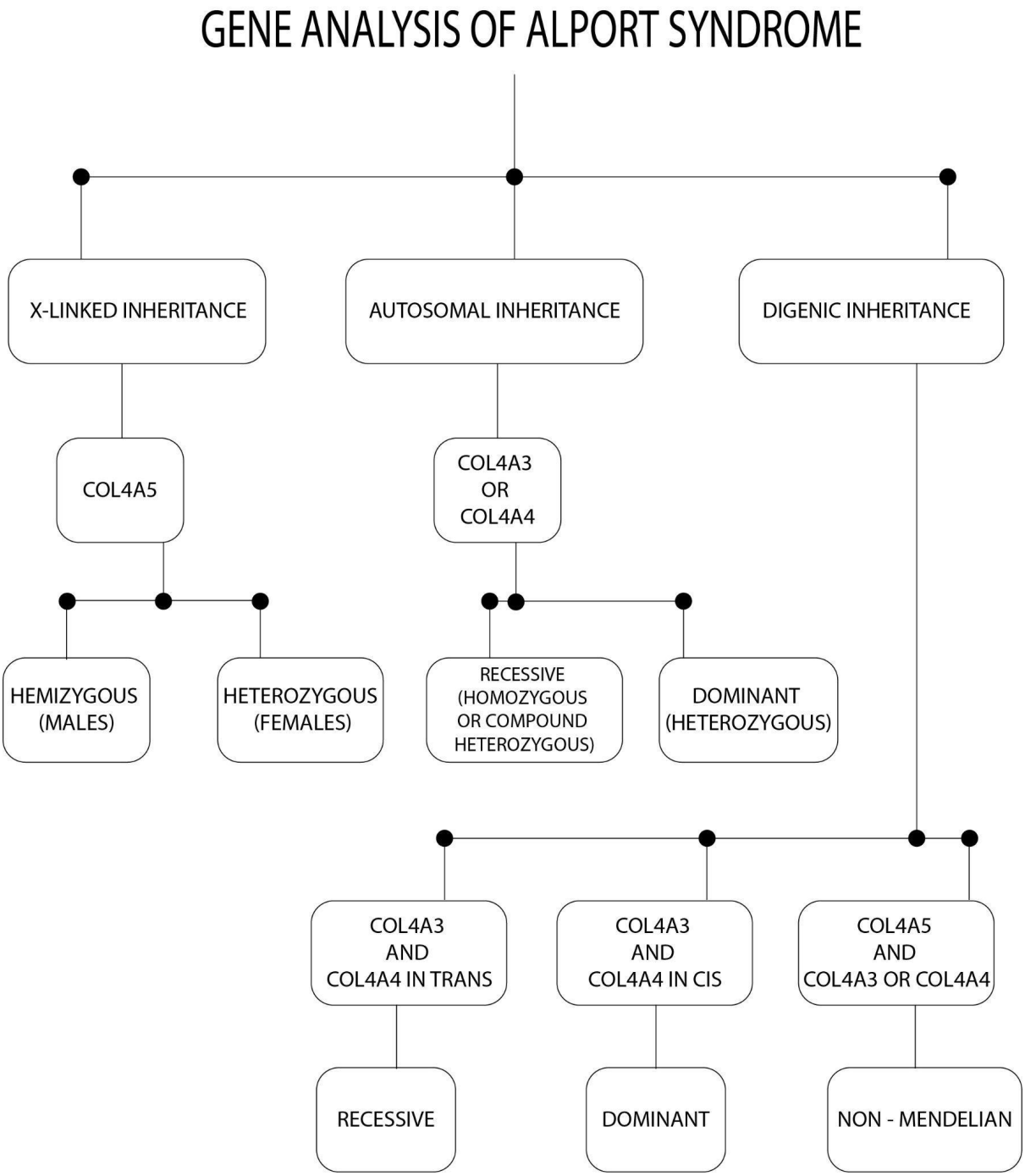

Figure 1.

Patients with AS exhibit a wide range of phenotypic variability, from progressive renal disease to isolated hematuria. The severity of renal manifestations in XLAS differs between males and females. All male patients with XLAS develop proteinuria and eventually progress to renal insufficiency. In contrast, female patients typically present with milder and more variable symptoms, ranging from isolated hematuria to ESRD, with later onset and slower

disease progression. [12] The GBM is composed of multiple triple helices formed by α3(IV), α4(IV), and α5(IV) collagen chains. Pathogenic variants in the encoding genes disrupt the structure of one or more of these α chains, causing GBM deterioration. This deterioration leads to splitting of the lamina densa and the characteristic "basket weave" appearance. These structural changes accelerate glomerular sclerosis, ultimately impairing kidney function. In XLAS, pathogenic variants in the COL4A5 gene, which encodes the α5(IV) chain, are responsible for the disorder.

This genetic alteration can lead to two outcomes: either a complete absence or reduction of the α5-chain protein, or the production of a full-length protein with amino acid substitutions or additions. In the first scenario, an incomplete protein is typically non-functional, resulting in the development of disease. In the second scenario, amino acid substitutions or additions can cause localized bending or improper folding of the protein, disrupting the formation of the triple helix structure. Additionally, abnormally folded proteins are more sensitive to proteases, making them highly susceptible to degradation. [13]

The diagnosis of AS is primarily based on glomerular biopsy ultrastructure, clinical criteria, family history, and genetic testing, with an emphasis on early identification to delay or prevent ESKD. [9,14–16] AS is a monogenic disorder, meaning molecular testing can provide a definitive diagnosis when a pathogenic DNA variant is detected, potentially eliminating the need for invasive renal biopsies. Experts recommend genetic testing in cases of persistent familial microscopic hematuria. Additionally, for individuals with persistent proteinuria and steroid-resistant nephrotic syndrome, genetic testing for COL4A genes is advised to rule out inherited focal segmental glomerulosclerosis (FSGS) and unexplained kidney failure. [17]

Accurate differentiation of AS from other glomerulopathies is critical to avoid inappropriate immunosuppressive therapies and to initiate renoprotective treatments promptly. Several landmark clinical trials have demonstrated the efficacy of pharmacological interventions targeting the renin-angiotensin-aldosterone system (RAAS) blockade. [4,18]

In the pursuit of precision medicine, genetic testing remains the gold standard for diagnosing AS due to its superior sensitivity and specificity compared to kidney biopsy. [19] However, molecular testing may not always resolve diagnostic challenges, highlighting the need for a comprehensive approach that combines genetic insights with clinical evaluation. [17]

Genetic testing plays a crucial role in confirming diagnoses, particularly in cases of Autosomal Recessive AS (ARAS) or when clinical symptoms overlap with other kidney disorders. Among the available methods, whole exome sequencing is often preferred because

it enables simultaneous analysis of all coding regions. [10,20] Advances in whole-genome and whole-exome sequencing are helping to identify rare variants, which enhance genetic testing and improve genotype–phenotype correlations. [15]

A strong correlation between genotype and clinical manifestations is especially evident in males with XLAS. Males with a hemizygous mutation typically present with the full spectrum of renal and extrarenal features associated with the disease. These individuals often experience a more severe disease course, with early-onset ESRD and deafness, as highlighted in the discussed cases. Conversely, females with XLAS, being heterozygous, exhibit a broader range of clinical symptoms and disease progression. This variability is believed to result from X-inactivation mechanisms in females. [21] The severity of the disease in XLAS is not directly linked to the type of mutation, though certain mutations, such as large deletions, rearrangements, frame-shift mutations, and nonsense mutations, are associated with more severe disease presentations and an earlier onset of symptoms. [22] According to Kashtan et al., males with XLAS face varying risks of ESRD by the age of 30, depending on the mutation type: a 90% risk with deletion or nonsense mutations, 70% with splicing mutations, and 50% with missense mutations. [23] In the case discussed, the observed nonsense mutation likely accounts for the early development of nephropathy and hearing loss.

Previous genotype–phenotype correlation studies have shown that males with large deletions, frameshift mutations, or truncating variants in the COL4A5 gene tend to exhibit more severe symptoms and an increased likelihood of kidney failure at a younger age. Conversely, males with missense variants generally display milder disease features. [19] For females with heterozygous COL4A5 variants, no definitive genotype–phenotype correlation has been established regarding their age at kidney failure. However, females with missense variants in COL4A5 often retain better kidney function and have a lower likelihood of developing proteinuria compared to those with other types of variants. [22]

To date, hundreds of unique variants have been identified in the COL4A3-5 genes, which range in size from 150 kb to 250 kb. Moreover, the complex processes involving type IV collagen's triple-helix extracellular suprastructure formation, function, and degradation remain poorly understood. This complexity poses challenges in predicting an AS patient's clinical trajectory based solely on genetic test results. [16]

Alport Syndrome Treatments: Future Genomic Strategies

Currently, there is no cure for AS. Conventional pharmacological treatments have proven inadequate over the years as they primarily aim to slow the progression of symptoms rather than address the underlying cause of this debilitating condition. [16] Most AS treatment strategies involve the use of ACEIs and ARBs. [4] In recent years, there has been a growing focus on the research and development of treatments for CKD, including AS. Several investigational therapies are in development, such as sodium/glucose cotransporter 2 (SGLT2) inhibitors, [24] aminoglycoside analogs, [25] endothelin type A antagonists, [26] lipid-modifying agents, [27] hydroxychloroquine, [28] antimiR-21, [29] bardoxolone methyl, [30] and gene replacement therapies. [10]

GT offers a promising avenue for addressing diseases currently untreatable by conventional methods. GT involves the use of genetic material to treat or prevent diseases by targeting and modifying specific genes within cells using precise techniques. This can include altering gene functions or implementing gene-editing programs within cells to enable internal genetic modifications. [31] The potential of GT lies in its capacity to treat a wide range of diseases and disorders. [32]

For AS, experimental genome editing therapies aim to repair defective genes. These approaches may involve techniques such as introducing protective mutations, inactivating harmful mutations, modifying viral DNA, or delivering therapeutic transgenes. [33] Effective implementation of GT for AS requires the replacement of mutant alleles with corrected copies of the gene. This process involves delivering the normal gene to target tissues using a vehicle, such as a virus or nanoparticle. Once the corrected gene replaces the defective one, the modified cells can proliferate and produce sufficient quantities of the desired protein, restoring a normal phenotype. [34]

The genetic basis of AS lies in mutations in the COL4A3, COL4A4, or COL4A5 genes, which disrupt the synthesis and proper folding of the α3/α4/α5 peptide chains. This results in defective COL4α3α4α5 trimers that are unable to support the type IV collagen network. The ultimate goal of GT is to introduce healthy versions of these genes into podocytes, enabling the production of functional peptide chains that can form normal COL4 and restore the GBM. An alternative GT approach focuses on reducing the production of defective α3/α4/α5 peptide chains through DNA or RNA transduction to mitigate cellular inflammation and damage. 35]

Below, we explore some therapeutic strategies currently under investigation.

Gene editing by CRISPR/Cas9

Originally derived from a bacterial immune system, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system has evolved into a cutting-edge gene-editing tool with potential therapeutic applications for various rare genetic disorders. [36] Over time, numerous variants of the Cas9 nuclease have been identified and studied. [37,38] The CRISPR/Cas9 system relies on two main components: a single-guide RNA (sgRNA) and the Cas9 endonuclease. The sgRNA directs Cas9 to the target genomic site, enabling the creation of a precise double-strand break (DSB) via Watson-Crick base pairing. Typically, these DSBs are repaired through non-homologous end joining, which can lead to insertions or deletions. However, the precision of the repair process can be enhanced by introducing a donor DNA repair template compatible with the target region, allowing for the synthesis of wild-type DNA. [39]

A recent study by Daga et al. demonstrated the feasibility of using podocyte-lineage cells derived from urine to replicate the physiological conditions of these cells, enabling accurate evaluation of COL4 variant corrections following experimental interventions. This innovative approach was tested on two hereditary forms of XLAS and autosomal dominant variants using a self-inactivating dual-plasmid system. One plasmid induced DNA damage through a self-cleaving Streptococcus pyogenes Cas9 (SpCas9) paired with an sgRNA under the CMV promoter, while the other carried a double-stranded DNA (dsDNA) donor fragment for homologous repair. This method achieved high correction rates, with 44% for the COL4A3 gene and 58% for the COL4A5 gene, alongside reduced rates of indels (10.4% for COL4A3 and 8.8% for COL4A5).

In AS, the number of podocytes decreases over time due to structural defects that lead to apoptosis and cell death. CRISPR/Cas9 gene therapy is expected to be most effective in the early stages of the disease when restoring a functional GBM is still possible. While these results are promising, significant challenges remain before this proof of concept can be transitioned to in vivo studies, particularly due to the difficulties associated with manipulating podocytes. [40]

Jeff H. Miner introduced two innovative approaches for AS treatment: pro-repair gene activation (CRISPRa) to achieve gene gain-of-function and pathogenic gene deactivation or

inhibition (CRISPRi) to achieve gene loss-of-function. These strategies differ from the traditional CRISPR/Cas9 method, which focuses on directly repairing the genetic variants causing AS. Notably, neither CRISPRa nor CRISPRi requires DNA cleavage.

CRISPRa utilizes a catalytically inactive mutant form of Cas9 (dCas9) with its endonuclease activity disabled through point mutations. This dCas9 is fused with a transcriptional activator domain to regulate gene expression. A guide RNA (gRNA) directs the CRISPRa complex to a specific genomic locus, where instead of cutting the DNA, it activates downstream gene expression. [41] The rationale behind CRISPRa in AS treatment is based on the observation that in ARAS, the COL4A5 and COL4A6 genes remain intact. By activating the COL4A6 gene in podocytes, CRISPRa could induce the production and secretion of α5α5α6(IV) collagen. This heterotrimer could restore the GBM structure and function, compensating for the altered or absent α3α4α5(IV) collagen.

On the other hand, CRISPRi fuses dCas9 with a transcriptional repressor. When guided to a specific genomic locus by gRNA, this complex represses downstream gene expression. In the context of AS, where laminin subunit α2 deposition in the GBM damages podocytes and exacerbates glomerular disease progression, CRISPRi-mediated inactivation of the LAMA2 gene in glomerular cells could potentially slow disease progression. [40]

Lin et al. carried out a study using a COL4A3−/− mouse model of AS combined with an inducible transgene system. Their findings revealed that the secretion of α3α4α5(IV) heterotrimers by podocytes into a defective GBM successfully restored the missing collagen IV network. This intervention slowed the progression of renal disease and extended the mice's survival. [42]

In a related study, Funk et al. attempted to increase the expression of the COL4A3 transgene in endothelial cells of COL4A3−/− Alport mice using adenovirus-mediated gene transfer. However, the absence of COL4A3/A4 proteins or assembled α3α4α5(IV) heterotrimers in these mice hindered the resolution of the disease phenotype. [43]

Exon-skipping therapy using an antisense-oligonucleotide

A recent study highlights the development of an exon-skipping therapy using antisense oligonucleotides (ASOs) to treat severe cases of male XLAS. This therapy works by converting a truncating mutation into an in-frame deletion mutation at the RNA level, which

is generally less harmful. ASOs achieve this by binding to exonic splicing enhancer regions, disrupting exon splicing and facilitating exon skipping.

The study specifically targeted truncating variants in exon 21 of the COL4A5 gene and conducted a series of experiments, including a type IV collagen α3/α4/α5 chain triple helix formation assay, as well as in vitro and in vivo evaluations of treatment efficacy. Results showed that exon skipping enabled the formation of functional trimers, leading to significant clinical and pathological improvements. This included the restoration of α5 chain expression on the glomerular and tubular basement membranes. Furthermore, mice treated with ASOs demonstrated a noticeably prolonged survival period. These findings suggest that exon skipping may offer a promising therapeutic strategy for addressing severe cases of male XLAS.

The study also drew parallels between XLAS and Duchenne muscular dystrophy (DMD), both of which exhibit strong genotype-phenotype correlations, making them suitable for exon-skipping therapy. Although Eteplirsen, an ASO therapy for DMD, showed weaker than expected activity due to rapid filtration into the urine from the glomerulus and limited delivery to skeletal muscle, this challenge highlights the potential efficacy of developing ASOs specifically for kidney diseases. This report underscores the promising effects of ASO therapy for male XLAS and its potential as a novel treatment approach. [44]

A subsequent study examined the effects of exon-skipping therapy using kidney organoids generated from AS patient-derived induced pluripotent stem cells (AS-iPSCs). These organoids, which were developed from AS-iPSCs, demonstrated nephron-like structures. As anticipated, the C-terminus of COL4A5 was absent in the AS-derived organoids. However, ASOs successfully restored the expression of the COL4A5 C-terminus in vitro.

To further explore the therapeutic potential, the AS-organoids were transplanted into mice to evaluate GBM formation in vivo. The study observed that AS-organoids formed a lower slit diaphragm ratio compared to control organoids. Finally, the researchers assessed the impact of exon skipping on transplanted organoids but observed only minimal effects in this context.

These findings suggest that kidney organoids derived from AS-iPSCs can replicate the lack of COL4A5's C-terminus and that exon-skipping therapy can restore its expression in vitro. However, the therapy's effectiveness in vivo remains limited. [45]

Premature termination codon readthrough therapy

In AS, shortened α3, α4, and α5 collagen IV chains lack the NC1 domain due to nonsense mutations, preventing the formation of heterotrimers vital for GBM function. Additionally, nonsense-mediated mRNA decay reduces mRNA stability and expression levels. Readthrough agents, which bypass stop codons on the RNA level, have emerged as a potential therapy for AS patients with nonsense mutations. Recent screening of 49 COL4A5 nonsense mutations in AS patients revealed that 11 mutations were susceptible to readthrough therapy induced by G418, an aminoglycoside with strong readthrough activity. These findings suggest that readthrough therapy could be a viable treatment for some AS patients. [41]

Anti-miRNA-21 oligonucleotides

MicroRNAs (miRNAs) are short non-coding RNAs which can regulate gene expression by inhibiting the translation or increasing the degradation of their target messenger RNAs.
The ability of a single miRNA to regulate multiple downstream target mRNAs altered in disease conditions makes miRNAs attractive therapeutic targets with the potential to impact a variety of molecular pathways, [46] miRNA-21 has been found to be dysregulated in multiple kidney disorders, including AS. It was shown that renal miRNA-21 is upregulated in Col4α3−/−mice and the use of anti-miRNA-21 oligonucleotides significantly slows kidney disease progression and improves survival in Alport mice. [47] In patients with AS, the expression of miRNA-21 in kidney samples was found to be significantly higher compared to controls and it was correlated with severity of kidney disease as evidenced by proteinuria, kidney function biomarkers and renal pathology scores. [29]
Rubel et al. examined Col4a3−/− Alport mice serve as an animal model for renal fibrosis. MicroRNA-21 (miR-21) expression has been shown to be increased in the kidneys of AS patients. Here, the study investigated the nephroprotective effects of Lademirsen anti-miR-21 therapy. They used a fast-progressing Col4a3−/− mouse model with a 129/SvJ background and an intermediate-progressing F1 hybrid mouse model with a mixed genetic background, with ACEi monotherapy in combination with anti-miR-21 therapy. In the fast-progressing model, the anti miR-21 and ACEi therapies showed an additive effect in the reduction in fibrosis, the decline of proteinuria, the preservation of kidney function and increased survival.

In the intermediate- progressing F1 model, the anti-miR-21 and ACEi therapies individually improved kidney pathology. Both also improved kidney function and survival; however, the combination showed a significant additive effect, particularly for survival. RNA sequencing (RNA-seq) gene expression profiling revealed that the anti-miR-21 and ACEi therapies modulate several common pathways. However, anti-miR-21 was particularly effective at normalizing the expression profiles of the genes involved in renal tubulointerstitial injury pathways. In conclusion, significant additive effects were detected for the combination of anti-miR-21 and ACEi therapies on kidney function, pathology and survival in Alport mouse models, as well as a strong differential effect of anti-miR-21 on the renal expression of fibrotic factors. These results support the addition of anti-miR-21 to the current standard of care (ACEi) in ongoing clinical trials in patients with AS. [48]

Protein replacement therapy

Protein replacement therapy remains an attractive and unexplored opportunity for Alport patients. Delivery of full-length recombinant laminin molecules to the GBM [49] set up a possibility that a full-length or mini-α345 protomer can be delivered therapeutically to the glomerulus, where it can oligomerize forming the α345 scaffold in the GBM. Recent advancements in producing the α345 NC1 single-chain trimer [50] and a miniature version of α345 collagen IV protomer, named miniprotomer, provide tools to begin development of protein replacement therapy. Both trimers and miniprotomers may harbor sufficient activities to have a therapeutic effect. Further steps will include development and testing of full-length α345 protomers. [51]

Pharmacological chaperones

Pharmacological chaperones (pharmacoperones), originally defined as molecules that correct protein misfolding, have now been expanded to include molecules that stabilize the final protein structure. These "second-generation" pharmacoperones help facilitate the folding of aberrant variants and slow down protein degradation. The most effective class targets binding pockets that do not interfere with the protein’s function. This class of molecules has the potential to stabilize the α345 hexamer without disrupting its function, offering a new

therapeutic approach for AS. Our single-chain trimer technology enables high-throughput screening to identify potential drugs for AS. [52]

X-chromosome reactivation

One approach being explored for AS is X-chromosome (Xc) reactivation. In females, one of the X chromosomes is randomly inactivated during early development, ensuring balanced expression between XX females and XY males. The idea is to reactivate the healthy copy of the COL4A5 gene on the inactivated Xc in women with XLAS. However, this approach is still in the experimental stage, with concerns about potential off-target effects being a significant limitation. [5]

Difficulties Facing Gene Therapy

Research into GT for AS has faced challenges, particularly with vector selection. Adenovirus, although widely studied, triggers strong immune responses and has short-lived expression, limiting its clinical use. Lentivirus raises concerns about random genome integration, and while recombinant adeno-associated virus (rAAV) holds promise, its small gene load (4.7 kb, or 2.7 kb after excluding regulatory sequences) limits its ability to carry large genes like COL4A3/A4/A5, which are about 5.0 kb. Despite these challenges, progress is ongoing. Glomerular podocytes, which express and secrete the collagen IV trimer, are crucial for AS GT but are difficult to target due to the restrictive glomerular filtration barrier, which limits gene delivery efficiency. [54,55]

Despite challenges, there is hope for GT in AS. While intravenous administration may be less effective at reaching glomerular podocytes, higher viral doses can improve transduction efficiency. Professor Saleem Ma's team achieved successful gene transduction in podocytes using high doses of rAAV, improving kidney function and pathology in induced podocin knockout mice. However, higher doses triggered immune responses and non-target transduction, highlighting the need for better delivery methods. A new dual AAV system, which divides large genes into segments and recombines them in target cells, offers a solution to rAAV's capacity limitation. This system has shown promise in treating bilateral deafness in Otof−/− mice and demonstrated efficacy and safety in pediatric clinical trials. [56-60]

Conclusion

Early genetic diagnosis of AS is crucial for timely treatment to improve patient well-being and life expectancy. Sequencing techniques are now efficient, affordable, and the gold standard for diagnosing AS, offering greater sensitivity and specificity than kidney biopsy. Genetic testing also helps assess clinical course by determining the exact genotype. GT holds promise as a potential cure for this monogenic disorder, though challenges remain in effectively delivering therapeutic genes to renal target cells. Researchers are exploring various models, carriers, and methods to overcome these hurdles. Recent advances focus on deciphering pathogenic mechanisms behind collagen IV assembly, DNA editing tools, RNA splicing interference, and controlling mRNA translation. By leveraging successful GT strategies from other fields, targeted therapy could reverse AS, especially if applied early. Despite the high cost, technical challenges, and off-target effects, ongoing research into innovative treatments offers hope for AS patients. Collaborative efforts between clinicians and geneticists are essential to advancing GT for AS.

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Authors do not report any disclosures.

Author's contribution

All authors contributed to the article.

Conceptualization: MK, BP, MS, AM
Methodology: MK, BP
Software: BP, MS
Formal analysis: MK, MS, AM
Investigation: MK, AM
Data curation: BP, MS
Writing - rough preparation: MK, BP, MS, AM
Writing - review and editing: BP, AM
Visualization: MK, AM
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