Full Review

Alport syndrome from bench to bedside: the potential of current treatment beyond RAAS blockade and the horizon of future therapies

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ABSTRACT

The hereditary type IV collagen disease Alport syndrome (AS) always leads to end-stage renal failure. Yesterday, for the past 90 years, this course was described as ‘inevitable’. Today, RAAS blockade has changed the ‘inevitable’ course to a treatable disease. Tomorrow, researchers hope to erase the ‘always’ from ‘always leads to renal failure’ in the textbooks. This review elucidates therapeutic targets that evolve from research: (i) kidney embryogenesis and pathogenesis; (ii) phenotype-genotype correlation and the role of collagen receptors and podocytes; (iii) the malfunctioning Alport-GBM; (iv) tubulointerstitial fibrosis; (v) the role of proteinuria in pathogenesis and prognosis; and (vi) secondary events such as infections, hyperparathyroidism and hypercholesterolaemia. Therefore, moderate lifestyle, therapy of bacterial infections, Paricalcitol in adult patients with hyperparathyroidism and HMG-CoA-reductase inhibitors in adult patients with dyslipoproteinemia might contribute to a slower progression of AS and less cardiovascular events. In the future, upcoming treatments including stem cells, chaperon therapy, collagen receptor blockade and anti-microRNA therapy will expand our perspective in protecting the kidneys of Alport patients from further damage. This perspective on current and future therapies is naturally limited by our personal focus in research, but aims to motivate young scientists and clinicians to find a multimodal cure for AS.

Keywords: Alport syndrome, chaperon therapy, discoidin domain receptor 1, kidney fibrosis, microRNA-21

ESSENTIALS FOR FUTURE THERAPIES: PATHOGENESIS OF ALPORT SYNDROME AND KIDNEY EMBRYOGENESIS

Alport syndrome (AS) is a hereditary type IV collagen disease, which always leads to progressive renal fibrosis and end-stage renal failure [1]. Three different type IV collagen trimers are deposited in basement membranes: \( \alpha_1/\alpha_1/\alpha_2 \), \( \alpha_3/\alpha_4/\alpha_5 \) and \( \alpha_5/\alpha_5/\alpha_6 \) (IV) [2]. The mature glomerular basement membrane (GBM) predominantly contains \( \alpha_3/\alpha_4/\alpha_5 \) type IV collagen chains. Mutations in the type IV collagen genes \( COL4A3/4/5 \), which encode the \( \alpha_3/\alpha_4/\alpha_5 \) chains, cause AS. These mutations interfere with the correct assembly of the \( \alpha_3/\alpha_4/\alpha_5 \) (IV) collagen network in the GBM and hinder the developmental switch from the embryonic \( \alpha_1/\alpha_1/\alpha_2 \) (IV) network to the mature \( \alpha_3/\alpha_4/\alpha_5 \) (IV) network, causing the persistence of an immature GBM [3, 4]. Consequently, a thickening and splitting of the GBM in AS causes progressive renal fibrosis leading to end-stage renal failure [5]. Maturation of the GBM develops in the neonatal age; therefore, a child with AS is not born with an abnormal GBM (but develops AS during maturation of the GBM)—leaving a therapeutic
window for very early therapy. In fact, the defective Alport-GBM can be restored in mice [6].

**GENOTYPE-PHENOTYPE-CORRELATION POINTS TO THE ACHILLES’ HEEL IN ALPORT PATHOGENESIS: PODOCYTES AND THEIR COLLAGEN RECEPTORS**

The α3/α4/α5 type IV collagen chains are exclusively produced by podocytes [7]. The genotype-phenotype correlation in AS [8] points to a role of the α3/α4/α5 (IV) producing and sensing cells in AS pathogenesis: the podocytes sense their GBM via collagen receptors such as α1β1 and α2β1 integrins and discoidin domain receptor 1 (DDR1). All of these collagen receptors have been shown to influence Alport pathogenesis [5, 9, 10], rendering them as possible therapeutic targets [11].

**THE ROLE OF A SPONGY ALPORT-GBM FOR THERAPY**

The GBM in most AS patients consists of α1/α2 (IV) chains only, making this altered GBM more porous [12] (resulting in acanthocyte formation) and more susceptible to endoproteolysis [3]. The GBM in AS patients is thought to be more vulnerable by increased (or even normal) filtration pressure. Therefore, thickening and splitting of the GBM in AS in part is a stress response of the podocytes. Any medication reducing the mechanical stress on the podocyte, such as RAAS blockade, reduces the risk of GBM ruptures and focal segmental glomerulosclerosis, which is a common light microscopical glomerular feature in AS.

**THE ROLE OF TUBULOINTERSTITIAL FIBROSIS IN THE COURSE AND PROGNOSIS OF AS**

AS is a glomerular disease; however, tubulointerstitial fibrosis is the key feature in progressive renal damage leading to end-stage renal failure. For example, RAAS blockade is not able to hinder thickening and splitting of the GBM in AS, but markedly delays tubulointerstitial fibrosis [13]. The glomerular disease and the podocyte stress response lead to distribution and secretion of profibrotic chemokines and cytokines in the primary urine that are re-absorbed by the tubular cells. The re-absorbed profibrotic chemokines lead to tubular scar tissue formation that finally demolishes the kidney. Therefore, until now, the amount of tubulointerstitial fibrosis is the most accurate histological prognostic factor regarding the evaluation of kidney function.

**THE ROLE OF PROTEINURIA IN PATHOGENESIS AND PROGNOSIS OF AS**

Proteinuria reflects ongoing glomerular inflammatory damage in all autoimmune diseases such as lupus nephritis and most types of glomerulonephritis. Therefore, the amount of proteinuria serves as an important prognostic factor in order to evaluate response to therapy. In contrast, the amount of proteinuria is a poor prognostic marker in AS, because it does not automatically correlate with inflammation and scar tissue formation. For example, loss of 5 g protein per day (without TGFβ and CTGF) can have a better prognosis than ‘only’ 1 g per day with relatively high levels of profibrotic chemokines. We think that progression from haematuria to microalbuminuria and from microalbuminuria to overt proteinuria represent very important steps in the course of Alport disease. However, once the patient has reached the level of ‘overt proteinuria’, increasing amounts of proteinuria do not necessarily result in a worse prognosis or disease progression (unpublished data from the European Alport registry [13]). In contrast, there is increasing evidence that the quality and quantity of proinflammatory and profibrotic proteins in the urine determines the course of AS [14].

**THE UNDERESTIMATED ‘COLLATERAL DAMAGES’: RECURRENT INFECTIONS, HYPERPARATHYROIDISM, CARDIOVASCULAR DISEASE AND HYPERCHOLESTEROLAEMIA**

In mice with AS, bacterial CpG-DNA accelerates glomerulosclerosis by inducing a M1 proinflammatory macrophage phenotype and podocyte loss [15]. Vice versa, patients with ongoing renal failure are immuno-compromised and are more likely to get recurrent bacterial infections. Therefore, these bacterial infections should be treated rigorously and good dental health might contribute to a slower progression of AS. Most patients with progressive renal failure develop secondary hyperparathyroidism due to their impaired calcium-phosphate-vitamin D balance. In mice with AS, the vitamin D receptor activator Paricalcitol (but not Calcitriol) showed a synergistic nephroprotective effect on top of early ACE inhibition [16]. Paricalcitol is board-approved for therapy of secondary hyperparathyroidism. Therefore, in adult patients with AS and incipient hyperparathyroidism, Paricalcitol might be an additional treatment option to delay renal failure.

Young patients with chronic renal disease have a 1000-fold higher risk of cardiovascular effects compared with healthy subjects. Proteinuria in a nephrotic range causes hypercholesterolaemia. In mice with AS, the HMG-CoA-inhibitor cerivastatin (statin) prolonged the lifespan until renal failure and delayed uraemia [17]. These effects were associated with decreased renal fibrosis and a reduction of inflammatory cell infiltration. Statins are board-approved for therapy of dyslipoproteinemia. Therefore, in adult patients with AS and incipient hypercholes terolaemia, statins might be an additional treatment option to delay renal failure and prevent cardiovascular events.

Figure 1 summarizes the key features of possible therapies on top of RAAS blockade. All medications in Figure 1 are board-approved for other medical conditions, but all possible therapies are off-label in AS and are very likely to stay off-label in the future. Medications in Figure 1 do not represent an expert recommendation for therapy of AS, but summarizes...
medical therapies currently used (or could be used) in adult patients with AS.

**FUTURE THERAPIES: COLLAGEN IV PATHOLOGY AND IDEAS FOR NEW THERAPEUTIC APPROACHES**

Collagens are large triple-helical proteins participating in a variety of processes in connective tissues, including tissue scaffolding, cell adhesion and tissue repair, among others. Structurally, there are two major categories, the fibrillar and the non-fibrillar collagens. Prime examples of the first are the type I, II, III and V collagens that exert a fundamental gluing role in extracellular matrix, holding cells together; a prime example of the latter is type IV collagen, the most abundant component of all basement membranes (BM), with a crucial role in the kidney glomerular filtration barrier [18]. A critical characteristic relating to this review is the participation of fibrillar collagens in higher order structures, through multiple nucleation events, a process that in case of mutations is susceptible to strong dominant negative effects. This is exemplified in diseases such as osteogenesis imperfecta where heterozygous mutations in type I collagen [(α1)2, α2)] interfere with triple-helix formation, affecting 75% of molecules and delaying protein secretion. These defective helices interfere next with proper nucleation and fibril formation in bones and other connective tissues [19, 20]. One can anticipate that the more defective molecules are secreted, the worse the phenotype is going to be. Actually, it was shown that the phenotype was milder if a premature termination of translation prevented chain association and triple-helix formation, which in turn prevented secretion of defective molecules.

To the contrary, type IV collagen of BM participates in network formation which is not the result of molecular nucleation events. Certainly there are interactions with other BM macromolecules such as laminin, nidogen and proteoglycans but there is no extensive nucleation and therefore less drastic dominant-negative effects are expected. For some type IV collagen mutations we hypothesize that should it be possible to have more molecules secreted in the extracellular matrix and participate in the meshwork, the phenotype might be milder compared with no secretion at all. About 50% of AS patients inherit missense or small in-frame mutations in the X-linked COL4A5 gene, where mature protomers are either secreted normally or abnormally, based on immunostaining. Absent or weak staining is due to poor secretion, most probably because quality control in the ER recognizes and degrades the misfolded chains [21]. This is accomplished through the unfolded protein response (UPR) pathway which is activated by the ER stress. UPR activation aims at restoring homeostasis by promoting proper protein folding using chaperones. When this fails for various reasons, the unfolded or misfolded molecules are removed by degradation. Prolonged ER stress may lead to protein translation pause or even to podocyte apoptosis or death through other mechanisms and foot processes effacement. Mechanisms aimed at enhancing the intracellular chaperone machinery or externally administered small chemicals that mimic chaperones could promote triple-helix formation and consequently allow hypomorphic mutants to exert their function, even though not perfectly, once found in the BM. This has been shown in numerous other occasions, such as cystic fibrosis and nephrogenic diabetes insipidus [22, 23].

A most recent example relating to BM pathology was the transgenic expression of the mutant rat C321R-LAMB2 gene in Lamb21/2 mice, a model that recapitulates Piersonian phenotypes and which has been used to study the role of LAMC2 mutations in aortic aneurysms [24].

**FIGURE 1:** Medical therapies currently used (or could be used) in adult patients with AS and risk factors that might negatively contribute to progression of disease. Medications do not necessarily represent an expert recommendation for therapy of AS.
syndrome. This arrangement attenuated the severe proteinuria, while it caused ER stress, noting that mutant protein was better than no protein. Also, the same authors showed that the use of chemical chaperone taurodeoxycholic acid in cells facilitated protein folding and trafficking and greatly increased secretion of the mutant LAMB2 [24]. Previously, Ohashi et al. had shown that mutant podocin due to NPHS2 mutation p. R138Q could not reach its destination in plasma membrane of cultured cells due to retention in the ER. Incubation with chemical chaperones caused folding of the mutant podocin and redistribution to the plasma membrane [25]. With regard to collagens, Murray et al. showed recently that treatment with the chemical chaperone 4-phenyl butyric acid reduced intracellular accumulation of mutant collagen IV in cultured patient primary cells. This ameliorated the cellular phenotype of a COL4A2 mutation that caused haemorrhagic stroke [26]. It is tempting to hypothesize that once found in its correct destination the mutant protein will function properly or near properly, depending on the exact nature of the mutation. Hypomorphic and milder mutations that interfere with molecule folding may be selected against during a very strict quality control early on in the ER; however, it is reasonable to hypothesize that once folded with the accessory contribution of external chaperones, these molecules may reach their destination and function sub-optimally but adequately and prevent severe disease progression.

Recently we created a new AS knockin mouse model, carrying missense mutation COL4A3-G1332E that demonstrated UPR activation in glomeruli. Hopefully, this will serve as a tool for testing a variety of available chemical chaperones and other alternative therapeutic approaches for alleviating or halting disease progression [27].

**FUTURE THERAPIES: STEM CELL-BASED THERAPIES FOR AS**

Stem cells and renal progenitors might offer a possible novel treatment of AS. Being able to deliver to the affected glomeruli a cell that could potentially become a mature podocyte, and produce new functional GBM, could be considered the ‘Holy Grail’ in the treatment of AS. Even if different groups [28–30] have claimed podocyte differentiation from stem cells in Alport mice, these publications still need validation and do not convincingly demonstrate that podocyte differentiation and consequent restoration of the GBM really occurs. In fact, the concept that direct differentiation of stem cells into organ-specific mature cell types occurs and can rescue the progression of disease has almost been abandoned; stem cell integration and differentiation is a very rare event that cannot sustain the complete regeneration of kidney [31] or other organs.

In particular, our group has demonstrated [32] that differentiation of injected stem cells (amniotic fluid stem cells) into podocytes does not occur in vivo in an animal model of AS, despite significant protection of glomerular structure and function. The main mechanism of action of stem cells appears to be via paracrine activity (possibly affecting the TGFβ axis) leading to attenuation of fibrosis and chronic inflammation [32–34], in addition to stimulating ingress of ‘healing’ type II macrophages (M2) [34]. In addition, stem cells seem to induce blockade of the angiotensin II pathway that prevents further damage to the glomeruli and favours preservation of podocyte number. Thus, based on studies published so far, cell-based therapies have the potential to slow but not prevent renal injury in AS. Despite these encouraging results and increases in the lifespan of treated mice, the restoration of a functional GBM is still the elusive but requisite goal to actually cure AS [6].

In continuing efforts to find a cell source that produces the proper GBM, we and others have been working to obtain ‘new podocytes’ including in the form of nephron progenitors that can be induced to become podocytes. Several studies conducted on the differentiation of embryonic stem cells (ESC) and adult stem cells into intermediate mesoderm or cap mesenchyme (embryological tissues from which the kidney originates) have demonstrated the possibility of inducing committed cell differentiation in renal lineages [6, 35–38]. Other groups [39] have demonstrated the possibility of expanding nephron progenitors using induced pluripotent stem cell (IPS) technology, while Song et al. [40] have a newly developed IPS line of cells that ‘resemble’ in vivo podocytes. However, generation of differentiated renal structures from IPS or ESC has very low efficiency and is probably insufficient for strategies to directly translate these cells into potential therapeutic agents [41]. We have recently reported the isolation of a subpopulation within the human amniotic fluid that possess characteristics of nephron progenitors and that can be differentiated into podocytes expressing the collagen IV trimer [42].

With the challenges of fibrosis, chronic inflammation and podocytes incapable of laying down the proper type IV collagen network, a cell-based approach would ideally be able to act simultaneously on all these aspects in order to offer a robust treatment for AS. In this regard, different cell-based strategies might be combined together. Stem cells, being more immune-privileged than adult renal cells, can be systemically infused and act in reducing fibrosis and inflammation, while direct replenishment of new non-defective podocytes derived from nephron progenitors might be the solution to replace the failing membrane (Figure 2). Nevertheless, one of the biggest challenges will be getting the cells across the GBM. An important feature of the novel podocyte progenitor cells mentioned above is their potential use in the development of small molecule-based therapies [6, 43] aimed at salvaging disturbed type IV collagen production. Thus, although we are still far from being able to harvest novel cell sources like stem or progenitor cells to support new therapeutic approaches, several promising avenues seem possible.

**FUTURE THERAPIES: COLLAGEN IV RECEPTOR BLOCKADE IN AS**

In general, the GBM structure is maintained by an equilibrium of synthesis and degradation. In Alport pathogenesis, increased synthesis of defective α3/4/5 type IV collagen, immature α1/α1/α2 type IV collagen and other basement membrane components in the GBM results in excessive
accumulation of matrix proteins [5]. Ablating the function of collagen receptors such as Discoidin domain receptor 1 (DDR1), integrin α1β1 or α2β1 might hinder or slow down podocytes’ matrix accumulation in AS [9, 10]. For example, loss of DDR1 reduced proinflammatory, profibrotic cells via signalling of TGFβ, CTGF, NFκB and IL-6 and decreased deposition of extracellular matrix. Immunogold staining and in situ hybridization identified podocytes as key players in DDR1-mediated fibrosis and inflammation [10].

This supports our hypothesis that podocyte–matrix interaction via collagen receptors plays an important part in progression of fibrosis. We postulate that specific receptor blockers such as DDR1 blockers might become a new therapeutic option in patients with AS in the near future.

**FUTURE THERAPIES: ANTI-MICRORNA THERAPY IN AS**

MicroRNA-21 (miR-21) has been shown to play a distinct role in the progression of kidney scarring in different animal models and humans [44]. Mice treated with anti-miR-21 oligonucleotides suffered far less interstitial fibrosis in response to kidney injury. Analysis of gene expression profiles identified groups of genes involved in metabolic pathways, including the lipid metabolism pathway regulated by peroxisome proliferator-activated receptor-α (Pparα), a direct miR-21 target [44]. Further, miR-21 sponge inhibited TGFβ-stimulated phosphorylation of Akt kinase, resulting in attenuation of phosphorylation of different Akt substrates that regulate mesangial cell hypertrophy [45]. Additionally, inhibition of miR-21 reduced TGFβ-stimulated fibronectin and collagen expression [45]. TGFβ is a very well-known key player in Alport pathogenesis and progressive kidney fibrosis in AS [13].

These studies demonstrated that miR-21 contributes to fibrogenesis and is a promising candidate target for antifibrotic therapy in AS. We postulate that anti-miR-21 compounds might become a new therapeutic option in patients with AS in the near future.

Additional potential future targets of nephroprotective therapy in AS such as the complement system, chemokine receptor blockade, TNFα-blockade and inhibition of matrix-metalloproteinases are summarized in a recent review [11].

**PERSPECTIVE CURRENT AND FUTURE THERAPY**

Yesterday, in the textbooks the course of AS was described as ‘inevitable’ for the past 90 years after the first description by Alport [46].

Today, RAAS blockade has changed the ‘inevitable’ course of AS to a treatable disease [47, 48] and led to treatment recommendations [49, 50]. Our review summarized additional therapies that are currently used in humans and that might influence the course of AS (Figure 1). There is preliminary scientific evidence for effectiveness in animal models. However, the long-term effects of these therapies still need to be evaluated in humans with AS. These therapies might well be additive to RAAS blockade and might further delay renal failure by years. Some medications are already board-approved for other indications; therefore, international Alport registries hopefully will generate the evidence for patients with AS.

Tomorrow, our review draws a bright horizon of several promising new therapies, all with different targets additive to existing therapies. This revives hope that AS has not only become a treatable disease, but end-stage renal failure can be prevented in most patients by multimodal therapy.
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CONFLICT OF INTEREST STATEMENT

None declared.

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