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TOPICAL REVIEW

Alport syndrome: its effects on the glomerular filtration barrier and implications for future treatment

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Abstract The glomerular filtration barrier comprises a fenestrated capillary endothelium, glomerular basement membrane and podocyte slit diaphragm. Over the past decade we have come to realise that permselectivity depends on size and not necessarily charge, that the molecular sieve depends on the podocyte contractile apparatus and is highly dynamic, and that protein uptake by proximal tubular epithelial cells stimulates signalling and the production of transcription factors and inflammatory mediators. Alport syndrome is the second commonest monogenic cause of renal failure after autosomal dominant polycystic kidney disease. Eighty per cent of patients have X-linked disease caused by mutations in the *COL4A5* gene. Most of these result in the replacement of the collagen IV $\alpha3\alpha4\alpha5$ network with the $\alpha1\alpha1\alpha2$ heterotrimer. Affected membranes also have ectopic laminin and increased matrix metalloproteinase levels, which makes them more susceptible to proteolysis. Mechanical stress, due to the less elastic membrane and hypertension, interferes with integrin-mediated podocyte–GBM adhesion. Proteinuria occurs when urinary levels exceed tubular reabsorption rates, and initiates tubulointerstitial fibrosis. The glomerular mesangial cells produce increased TGFβ and CTGF which also contribute to glomerulosclerosis. Currently there is no specific therapy for Alport syndrome. However treatment with angiotensin converting enzyme (ACE) inhibitors delays renal failure progression by reducing intraglomerular hypertension, proteinuria, and fibrosis. Our greater understanding of the mechanisms underlying the GBM changes and their consequences in Alport syndrome have provided us with further novel therapeutic targets.

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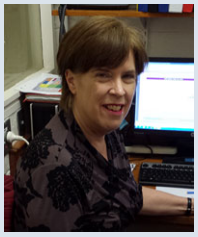
Abbreviations ACE, angiotensin converting enzyme; CTGF, connective tissue growth factor; DDR1, discoidin domain receptor 1; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; GBM, glomerular basement membrane; MMP, matrix metalloproteinase; TGFβ, transforming growth factor-β; TNFα, tumour necrosis factor α.

Judy Savige trained as a physician specialising in nephrology at the University of Melbourne and the Royal Melbourne Hospital. She completed her PhD in immunology at the Hammersmith Hospital laboratory and returned to a post-doctoral position at the Royal Melbourne Hospital and the Howard Florey Institute. Subsequent positions include Professor of Medicine at the University of Melbourne and Foundation Professor, Chairman of the Division of Medicine, and Director of Clinical Training at Northern Health. She has recently returned to the Royal Melbourne Hospital as a Professorial Fellow. Professor Savige's research interests have been inherited renal disease, especially Alport syndrome and Thin basement membrane nephropathy, the demonstration of ocular abnormalities in inherited renal disease and mutation databases. Her laboratory demonstrated the genes affected in thin basement membrane nephropathy and that this represented the carrier state for autosomal recessive Alport syndrome. Her laboratory co-curates the mutation databases for Alport syndrome with the Guy's Genetics laboratory, and for Dense deposit disease.



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Overview of glomerular filtration barrier

The nephron is the basic structural and functional unit of the kidney, and comprises the glomerulus, proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct. The glomerulus consists of capillary loops from which water and low molecular weight molecules (glucose, amino acids, creatinine, urea) move across the filtration barrier into Bowman's space, and then into the proximal tubular lumen. Normally humans produce 180 L of filtrate daily from plasma with 10 kg protein, but less than 1 g of protein is found in the urine (Hausmann *et al.* 2010). Proteinuria is a risk factor for progressive renal failure in all forms of kidney disease, and tubulointerstitial inflammation, fibrosis and tubular atrophy correlate directly with the amount of proteinuria.

The filtration barrier consists of a fenestrated capillary endothelium; the glomerular basement membrane (GBM); and the slit diaphragm between podocyte foot processes (Fig. 1). The endothelial fenestrations and glycocalyx allow direct access of plasma proteins to the GBM (Kriz, 2008), the GBM results from the fusion of the basement membranes produced by the endothelial cells and podocytes (Abrahamson *et al.* 2009), and the slit diaphragm is formed by the lateral interaction of proteins from adjacent podocyte foot processes. Podocytes enclose the outer surface of the glomerular capillaries with narrow 'foot processes' that interdigitate with those of neighbouring cells. Adjacent foot processes are linked to each other by the slit diaphragms. Podocytes also interact basally with the GBM through integrin–cell matrix interactions that signal to the cytoskeleton and help regulate the GBM architecture, and porosity (Suh & Miner, 2013; Fig. 2).

The glomerular filtration barrier prevents the passage of molecules based on their size, that is, their molecular weight and radius (Ohlson *et al.* 2000). The nature and

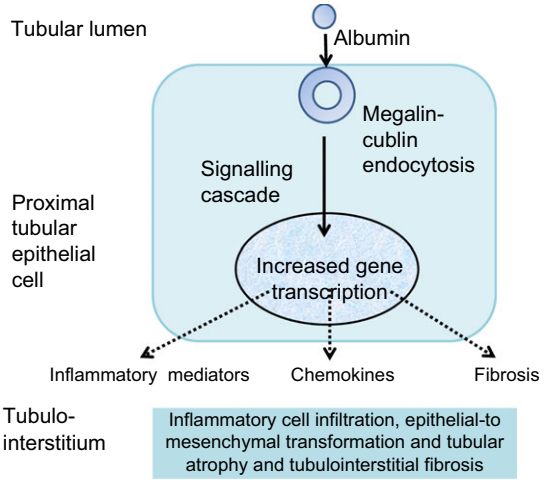

Figure 2. Pathogenesis of tubulointerstitial disease and disease progression in Alport syndrome
The figure is based on Noone & Licht, 2013.

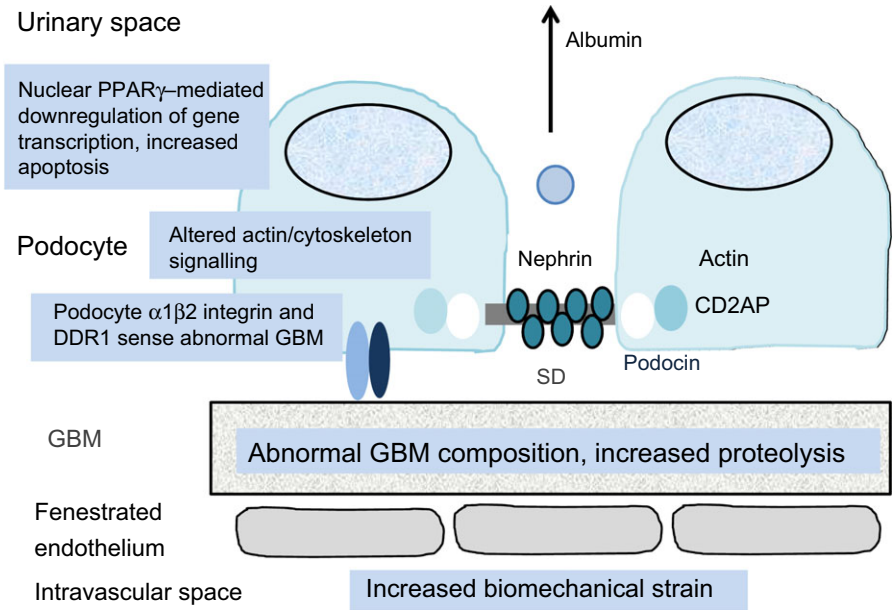

Figure 1. Factors in the pathogenesis of glomerular disease in Alport syndrome
A, increased biomechanical strain from hypertension, abnormal GBM, altered podocyte cytoskeleton and cell adhesion. *B*, lamellated or thinned GBM with different collagen IV and laminin molecules, and increased MMP proteolysis. *C*, podocyte $\alpha 2\beta 1$ integrin and DDR1 detect abnormal collagen IV. *D*, altered podocyte adhesion and actin/cytoskeleton. *E*, nuclear peroxisome proliferator-activated receptor γ (PPARγ) downregulation of SD slit diaphragm gene transcription and podocyte apoptosis (based on Noone & Licht, 2013).

location of the size barrier, and the relative contributions of the slit diaphragm, GBM and endothelium are not known. Whether the glomerular filtration barrier also selects for charge is controversial (Harvey & Miner, 2008).

Fenestrated endothelium

The endothelial fenestrae may contribute to protein sieving (Jeansson & Haraldsson, 2006), not through size since the fenestrae are too large, but possibly based on the negatively charged cell surface glycocalyx.

GBM

The GBM comprises mainly collagen IV, laminin, nidogen and the heparan sulphate proteoglycan, agrin. Collagen IV is a heterotrimer of α1–α6 chains. The individual chains all have non-collagenous termini and an intermediate triple helix with a Gly-Xaa-Yaa sequence (Hudson *et al.* 2003). Unlike fibrillar collagen, the collagen IV termini are retained and interact through their amino and carboxy ends via disulphide and sulfilimine bonds, and lysyl-oxidase-mediated cross-links (Hudson, 2004; Vanacore *et al.* 2009) to form a 'chicken wire-like' structure. Collagen IV is found in α1α1α2, α3α4α5 or α5α5α6 networks. The α1α1α2 network occurs in the GBM at birth but is replaced, in infancy, by the α3α4α5 heterotrimer in the glomerulus, cochlea and eye. Collagen IV is highly metabolically active with many binding partners (Parkin *et al.* 2011).

Laminin is a heterotrimer of α, β and γ chains, with variants of each of these chains producing at least 15 different isoforms (Miner & Yurchenco, 2004). Each molecule comprises a cruciform structure in which the N-termini of each of the three chains form a short arm and the carboxy termini combine to form a single long arm. The laminin trimers are named for their α, β and γ chains in this order, and four isoforms (laminin-111, -511 -521 and small amounts of laminin-332) are found in the kidney. Laminin-511 predominates in the GBM at birth but switches to laminin-521 in infancy when the collagen IV networks change. Laminin is linked to neighbouring podocytes by integrins and dystroglycan. The laminin–integrin interaction physically links the GBM to the podocyte actin cytoskeleton (through integrin and actin-associated proteins such as talin and α- actinin; Faul *et al.* 2007), and is reinforced by CD151.

Nidogen acts as a bridge between collagen IV and laminin, and probably stabilises the GBM. Agrin links laminin and the GBM to adjacent cells (Bezakova & Ruegg, 2003), but mice lacking agrin have no significant structural or functional defects (Suh & Miner, 2013).

Podocyte slit diaphragm

Podocyte foot process effacement and slit diaphragm abnormalities occur in nearly all cases of proteinuria. The significance of foot process effacement in the development of proteinuria is not clear.

The slit diaphragm consists of multiple layers of nephrin strands that connect adjacent podocyte foot processes (Kestila *et al.* 1998). This meshwork has channels that can accommodate albumin molecules (Wartiovaara *et al.* 2004). However, the slit diaphragm is located distally in the glomerular filtration barrier and is unlikely to be significant even if its pores are smaller than albumin (Jarad & Miner, 2009). Furthermore the amount of proteinuria does not correlate directly with slit diaphragm damage (Jarad & Miner, 2009).

Proteinuria

Tubular protein reabsorption

Albumin is the most abundant molecule in the glomerular filtrate and is reabsorbed by the proximal tubular epithelial cells. Excessive albumin loss in the filtrate and reabsorption by the proximal tubular cells results in abnormal signalling and a proinflammatory environment.

Proximal tubular epithelial cells have specific binding sites for albumin which is absorbed via receptor-mediated endocytosis. Megalin and cubulin form a complex that mediates albumin uptake. Megalin is a member of the lipoprotein receptor (LDL-R) family with a large extracellular domain and many complement-type, growth factor-repeats and an epidermal growth factor-repeat. The cytoplasmic tail has sequence motifs for phosphorylation probably for signalling. Cubulin, on the other hand, is almost entirely extracellular with only a small membrane anchoring domain.

For many ligands, megalin is processed via regulated intramembrane proteolysis (RIP). Phosphokinase C-dependent matrix metalloproteinase cleaves the receptor, a γ-secretase cleaves the remaining transmembrane–cytosolic receptor fragment, and the released fragment translocates to the nucleus where it upregulates gene transcription (Li *et al.* 2008). The cleaved megalin extracellular domain with its epidermal growth factor (EGF)-like structure binds to EGF-R and activates signalling.

Thus, excess urinary albumin results in a complex signalling cascade and upregulation of various chemotactic (MCP1, RANTES; Ninichuk *et al.* 2005), inflammatory (tumour necrosis factor α, TNFα) and profibrotic (transforming growth factor-β, TGFβ, and connective tissue growth factor, CTGF) mediators (Table 1; Baines & Brunskill, 2011). Albuminuria also appears to have a role in tubular cell apoptosis through

Table 1. Disease pathogenesis in Alport syndrome and targets of treatment

Mechanism	Strategy	Status as treatment and efficacy
Genetic mutation resulting in loss of collagen IV $\alpha3\alpha4\alpha5$ heterotrimer from the GBM	Correction of gene defect at genetic level	Not possible currently
	Nonsense mutations – inhibition of nonsense-mediated decay	No evidence yet
	Nonsense mutations – read through of mutation	Some evidence in other diseases such as Duchenne muscular dystrophy (Finkel *et al.* 2013)
	Stem cell therapy	Not possible currently in humans but appears to work in mice even with a damaged GBM
	Gene replacement therapy	Adenoviral vectors with LacZ expression cassette (Heikkila *et al.* 1996; Parpala-Sparman *et al.* 1999) and renal perfusion. Results in increased gene expression
	Bone marrow transplantation	Some expression of collagen IV $\alpha3\alpha4\alpha5$ and improved renal function in mice (Prodromidi *et al.* 2006; Sugimoto *et al.* 2006); improved renal function and life span but only marginally (LeBleu *et al.* 2009)
	Missense mutations – Chaperone therapy	Appears to work partially in cell lines (Pieri *et al.* 2014)
Mechanical strain	Renin–angiotensin–aldosterone blockade: Anti-hypertensive (reduces mechanical strain), antiproteinuric, antifibrotic	Efficacious in humans, delays onset of end-stage renal failure by up to 10 years (Gross *et al.* 2003)
	Aldosterone antagonist: antihypertensive and antiproteinuric	Reduces proteinuria and TGFβ1 (Giani *et al.* 2013)
	Other antihypertensives: reduce mechanical strain and renal failure progression	Less effective than ACE inhibition
Matrix metalloproteinase degradation of GBM	Inhibition of MMP-2, -3 and -9	Before proteinuria, delays onset of proteinuria and prolongs survival in mice (Zeisberg *et al.* 2006)
	Inhibition of MMP12 through CCR2	Prevents GBM damage in mice (Rao *et al.* 2006)
	Inhibition of MMP-12 through genetic ablation of USAG1	Inhibits disease progression and doubles lifespan in mice (Tanaka *et al.* 2010)
	Integrin $\alpha1$ chain knockout in podocytes and mesangial cells	Further protection against GBM destruction (Sayers *et al.* 1999)
	DDR1/2 inhibition	(Kruegel *et al.* 2013)
Inflammation	Chemokine receptor antagonists, reduce inflammation	May not be specific
	Irradiation, reduces neutrophil infiltration	Small improvement in renal function in mice (Katayama *et al.* 2008)
	Complement inhibitors	Inhibit proximal tubular epithelial cell production of inflammatory and profibrotic cytokines (Tang *et al.* 2009)
Fibrosis	TGFβ soluble receptor inhibitor	Minor improvement in normal mice; more pronounced when combined with $\alpha1$ blockade (Sayers *et al.* 1999)
	Reduced matrix accumulation, and expression of TGFβ1 and CTGF, through HMG-Co-A reductase inhibitors	Improves renal function and extends life span (Koepke *et al.* 2007)
	BMP7 antagonists potentially prevent tubular atrophy	No evidence yet *in vivo*

inhibiting the nuclear factor κB (NFκB)-dependent survival pathways (Takase *et al.* 2008).

There are also other proximal tubular cell albumin receptors including the neonatal Fc receptor and CD36 (Susztak *et al.* 2005; Roopenian & Akilesh, 2007)

Protein filtration

The last decade has seen two novel hypotheses proposed to explain the mechanisms underlying proteinuria (Smithies, 2003; Comper *et al.* 2008). The 'gel permeation/diffusion hypothesis' envisages protein diffusion through GBM behaving like a modified gel (Smithies, 2003). Increased protein concentration in the glomerular filtrate results from altered gel or GBM, from a reduced surface area for filtration with foot process effacement, or from reduced endothelial fenestration. These increase hydraulic resistance but diffusion of plasma proteins remains constant, that is, the same amount of protein is diluted in a smaller volume. The increased protein concentration in the filtrate then overwhelms tubular resorption. The more conventional explanation is that electrical forces determine glomerular permselectivity and there is evidence in non-mammalian systems for this (Hausmann *et al.* 2010).

The other novel mechanism describes a controversial route for tubular protein absorption. The conventional view is that small amounts of albumin are absorbed through the proximal tubule, that the rate of albumin take-up corresponds with fluid, and that most of the absorbed albumin is degraded and transported through the cells (Park & Maack, 1984). There is preliminary evidence that suggests more albumin leaks through the glomerular filtration barrier, and that much of this is returned to the circulation intact rather than being degraded (Comper *et al.* 2008).

Alport syndrome

Increasingly diseases are considered to affect the glomerular filtration barrier rather than any of its individual components. Alport syndrome represents one such condition.

Alport syndrome is an inherited form of progressive kidney failure with hearing loss and ocular abnormalities (Gubler *et al.* 1981; Savige *et al.* 2013). It affects one in 10,000 individuals, and 80% of families have X-linked inheritance while most of the others have autosomal recessive disease. X-linked disease is caused by mutations in the *COL4A5* gene (Barker *et al.* 1990), and autosomal recessive disease by two mutations in either the *COL4A3* or *COL4A4* gene. These genes code for the collagen IV α3–α5 chains. Collagen IV represents the major component of basement membranes in the glomerulus, cochlea, lens capsule and retina, which explains the clinical features. Heterozygotes for *COL4A3* or *COL4A4* mutations are carriers of autosomal recessive Alport syndrome, and have thin basement membrane nephropathy. This occurs in 1% of the population and is characterised by persistent isolated haematuria without proteinuria, hypertension or renal impairment. Hearing and ocular examination are normal.

Clinical features

Renal failure is the most significant risk of Alport syndrome, and males with X-linked inheritance develop end-stage disease before the age of 30 years ('early onset', usually with extrarenal features) or after 30 ('late onset', often with only hearing loss), and require dialysis or transplantation. Clinical features are generally milder in females with X-linked disease because of X chromosome inactivation, and about 15% develop renal failure by the age of 60 years (less than the published figure which was derived from hospital-based patients; Jais *et al.* 2003), and, rarely, the lenticonus or central retinopathy.

The GBM in Alport syndrome is lamellated, and there is secondary glomerulosclerosis and tubulointerstitial fibrosis. The rate of deterioration of renal function correlates better with the amount of tubulointerstitial fibrosis than with glomerulosclerosis (Hood *et al.* 2002).

Haematuria occurs through breaks in the GBM (Collar *et al.* 2001). The origin of the proteinuria is more complex. The observation that proteinuria occurs relatively late in Alport syndrome suggests that the abnormal collagen IV is not primarily responsible for any increased permeability (Abrahamson *et al.* 2007; Suh & Miner, 2013). In Alport syndrome, proteinuria occurs with the changes to the filtration barrier and, later, with compensatory hyperfiltration damage and glomerulosclerosis. This contrasts with Pierson disease where mutations in the laminin β2 gene produce proteinuria from birth (Pierson *et al.* 1963).

Hypertension is common in Alport syndrome too and poorly controlled hypertension increases the rate of renal failure progression.

All patients with Alport syndrome have a high tone sensorineural hearing loss that is helped with hearing aids. This is obvious from childhood in males with X-linked disease but plateaus in middle age. Hearing loss is also common in females with X-linked disease. The stria vascularis membrane in the cochlea is lamellated with an identical appearance to the GBM. The pathogenesis of the hearing defect is not well understood but the membrane appearance resembles that of the GBM and mechanical forces are probably important in both.

The ocular abnormalities do not affect vision significantly but are useful pointers to the diagnosis of Alport syndrome and its severity (Colville & Savige,

1997). The lenticonus or conical anterior protrusion of the lens eventually requires lens replacement and does not recur post-operatively. The central perimacular fleck retinopathy progresses until middle age but does not require treatment. Ultrastructurally the lens capsule is thinned and fractured, and the retinal membranes are thinned too.

What accounts for the differences in clinical features and outcome in Alport syndrome and thin basement membrane nephropathy? Individuals with thin basement membrane nephropathy have a heterozygous mutation in a collagen IV chain gene, and affected membranes are thinned rather than lamellated. The thinned membranes have reduced amounts of the collagen IV $\alpha3\alpha4\alpha5$ network but still sufficient that there is minimal replacement with the $\alpha1\alpha1\alpha2$ network, and probably no consequent increase in proteolysis, or mechanical stress. Thus both proteinuria and hypertension are uncommon in thin basement membrane nephropathy. Where proteinuria and renal impairment occur in Thin basement membrane nephropathy, it is likely that there is an additional mutation in the podocin gene (*NPHS2*; Savige *et al.* 2003; Tonna *et al.* 2008), a superimposed glomerular abnormality such as IgA glomerulonephritis, or the thinning is due to misdiagnosed X-linked Alport syndrome.

Diagnosis

The diagnosis of Alport syndrome is made with demonstration of a lamellated GBM on ultrastructural examination of the renal biopsy. In addition, there may be abnormalities in the collagen IV composition on immunohistochemical examination (Kashtan *et al.* 1989). In X-linked disease, the collagen IV $\alpha5$ chain, as well as the $\alpha3$ and $\alpha4$ chains are often absent from the GBM, and also from the epidermal basement membrane; and in autosomal recessive disease, the $\alpha3\alpha4\alpha5$ network is absent from the GBM but the $\alpha5$ chain persists in the $\alpha5\alpha5\alpha6$ network in the epidermis.

The recent *Expert guidelines on the diagnosis and management of Alport syndrome and thin basement membrane nephropathy* have indicated that genetic testing should be used to confirm the diagnosis of Alport syndrome (Savige *et al.* 2013). The advantages of genetic testing are that it makes the diagnosis with certainty, identifies the mode of inheritance, and, for X-linked Alport syndrome, the nature of the underlying mutation suggests the likelihood of early onset renal failure and the need for urgent treatment.

Genetic mutations

More than 1100 unique variants have been described in the *COL4A5* gene in X-linked Alport syndrome (https://grenada.lumc.nl/LOVD2/COL4A/home.php?select_db=COL4A5). Large deletions, rearrangements, small deletions and insertions, nonsense mutations, and carboxy terminus missense mutations usually produce early onset renal failure (Jais *et al.* 2000). Missense mutations at the amino terminus result in the later, adult onset disease, and splice mutations are associated with intermediate severity.

Nonsense mutations or changes that result in a downstream nonsense mutation account for 40% of all mutations in X-linked Alport syndrome, and missense mutations account for a further 40% (Hertz *et al.* 2012). Little is known about how these mutations cause disease, but in other collagen diseases, nonsense mutations result in nonsense-mediated decay, destruction of the collagen IV $\alpha5$ mRNA and loss of the corresponding $\alpha5$ chain from affected tissues (Bateman *et al.* 2009). Missense mutationis in other similar diseases result in the accumulation of misfolded collagen IV chains in the endoplasmic reticulum (ER) and their failure to be exported into the extracellular matrix (Pieri *et al.* 2014).

Thus, most mutations in X-linked Alport syndrome, at least in males, result in the loss of the collagen IV $\alpha5$ chain from affected tissues. The lack of any one of the collagen IV $\alpha3$, $\alpha4$ or $\alpha5$ chains results in the loss of the other chains which form the heterotrimer, and prevents its formation. There is a compensatory increase in the collagen IV $\alpha1\alpha1\alpha2$ network, which is usually a minor component on the subendothelial GBM surface. The resulting GBM is thinned but functions nearly normally for years until it becomes split, thickened and vacuolated.

Pathogenesis

Understanding the pathogenesis of Alport syndrome is the first step in developing effective treatments (Table 1) (Noone & Licht, 2013).

Disease initiation. Three interdependent mechanisms are responsible for the initiation of the typical Alport GBM abnormalities. The replacement of the collagen network with the $\alpha1\alpha1\alpha2$ heterotrimer is most important. The replacement network is more susceptible to biomechanical stress and to proteolysis (Kalluri *et al.* 1997). The collagen $\alpha1$ and $\alpha2$ chains have fewer cysteines than the $\alpha3$ and $\alpha4$ chains, and hence less cross-linking within and between heterotrimers (Zhou & Reeders, 1996; Gunwar *et al.* 1998). These chains also have more proteolytic cleavage sites (Parkin *et al.* 2011). Alport glomeruli also have increased levels of matrix metalloproteinases, MMP2, MMP-3, MMP-9 and especially MMP-12 (Zeisberg *et al.* 2006; Rao *et al.* 2006), probably secondary to hypertension-induced biomechanical strain (Meehan *et al.* 2009).

Secondly, the replacement of the normal collagen IV network with the $\alpha1\alpha1\alpha2$ heterotrimer results in the ectopic overproduction of laminins $\alpha1$ and $\alpha5$ and a disrupted architecture. Laminin-111 is increased in the regions of focally thickened GBM which are more permeable to protein (Abrahamson *et al.* 2007). Ectopic laminin deposition occurs through both podocycte signalling and mesangial cell activation. It further exacerbates the tendency to biomechanical strain and increases proteinuria.

Thirdly, the collagen IV $\alpha1\alpha1\alpha2$ network is less structurally sound and more susceptible to the mechanical strain caused by the intraglomerular hypertension and ultrafiltration. Biomechanical strain is present early since Alport glomeruli from preproteinuric mice are more deformable (Wyss *et al.* 2011). The normal glomerulus already operates under high hydrostatic pressure, but the pressures are even higher in Alport glomeruli because of the abnormal GBM, fewer cross-links and altered deformability. Pressures are also high within the cochlea, and together with the greater abundance of collagen IV $\alpha3\alpha4\alpha5$ in the kidney and ear, may explain why these tissues, but not the lung and small bowel, where they also occur, are affected.

Podocytes. The podocyte detects altered collagen IV through its $\alpha2\beta1$ integrin receptor. Integrins act as mechanosensors linking the extracellular matrix with the podocyte actin cytoskeleton, and a defective signal upregulates podocyte extracellular matrix deposition (Borza *et al.* 2012).

The podocyte also detects altered collagen IV through the discoidin domain receptor 1 (DDR1), a transmembrane tyrosine kinase receptor at the lateral margin of the podocyte foot processes between the GBM and slit diaphragm (Vogel *et al.* 1997), independent of integrin binding (Vogel *et al.* 2000). The podocyte upregulates levels of the cytokines and growth factors needed to repair the GBM, but these also increase inflammation and fibrosis, and hence disease progression (Gross *et al.* 2004, 2010).

The mechanical stresses on the adhesive interfaces between the podocytes and GBM, which are largely mediated through laminin-521–integrin $\alpha3\beta1$ interactions, further promote the accumulation of the abnormal laminins. Cell adhesion, cytoskeletal dynamics and cell signalling machinery are affected too (Cummins *et al.* 2007).

Mesangial cells, ectopic laminin deposition and cytokines. The mesangium is also important. Biomechanical stress on the glomerular capillary tuft initiates integrin $\alpha1\beta1$-mediated activation of Rac1/CDC42 and mesangial process invasion of the capillary loops (Zallocchi *et al.* 2013). These contribute to the deposition of laminin-211 which further exacerbates the mesangial invasion. The mesangial cytokines (TGF$\beta1$ and CTGF) and MMPs also contribute to the altered structural and functional properties of the Alport GBM with irregular thickening, splitting, and increased permeability. These events all affect podocyte cell health.

Proximal tubular epithelial cells, proteinuria, interstitial inflammation and fibrosis. Many cells within the kidney including podocytes, mesangial cells and proximal tubular cells produce chemokines (Sayyed *et al.* 2011), that attract leucocytes and macrophages to damaged tissue. Activated macrophages result in increased TNFα (Ryu *et al.* 2011), which is both pro-inflammatory and pro-apoptotic.

Matrix metalloproteinases are important in breaking down tubular basement membrane and allowing cells access to the tubulointerstititum (Cheng & Lovett, 2003).TGF$\beta1$ produced by podocytes activates fibroblasts, myofibroblast formation and epithelial-to-mesenchymal transition (EMT). The role of EMT is controversial but, while usually involved in repair, here results in fibrosis, with glomerulosclerosis and interstitial damage (Sayers *et al.* 1999). Complement activation appears to be important in EMT through TGF$\beta1$, since C3a receptor-deficient mice have less proteinuria, glomerulosclerosis and tubulointerstitial damage (Tang *et al.* 2009).

Disease progression. Mechanical strain, hypertension and proteinuria all contribute to disease progression in Alport syndrome (Meehan *et al.* 2009). Mechanical strain activates the rennin–angiotensin system (Durvasula *et al.* 2004) and induces SPARC (Secreted Protein Acidic and Rich in Cysteine; Durvasula & Shankland, 2005), both of which contribute to glomerulosclerosis. Hypertension accelerates proteinuria and induces matrix metalloproteinase production. TGFβ has a major role in disease progression and interstitial fibrosis. As the disease progresses and nephron mass is lost, glomerular hypertension further increases, exacerbating the biomechanical strain and the downstream disease mechanisms affected by it.

Treatment

Currently there is no specific treatment for Alport syndrome, and all males with X-linked disease and individuals with autosomal recessive disease eventually develop end-stage renal failure. However, it has become obvious that treatment with rennin–angiotensin blockade particularly before the onset of microalbuminuria slows down the rate of progression to end-stage disease (Gross *et al.* 2003, 2012). Renin–angiotensin blockade has many

potential actions in the treatment of Alport syndrome: blockade is antihypertensive, antiproteinuric and anti-fibrotic. A reduction in blood pressure puts less stress on the defective GBM, on the adjacent podocyte, and on the whole glomerulus (Wyss *et al.* 2011). Renin–angiotensin blockade is safe but only partially effective. Dialysis and renal transplantation in end-stage disease are effective and relatively safe, and potential new treatments must have greater efficacy and fewer risks than these approaches.

Is it possible to repair already established GBM defects? Recent experiments in a mouse model have confirmed that abnormal GBM can be repaired, for example, by the secretion of the wild-type collagen IV $\alpha 3\alpha 4\alpha 5$ heterotrimer, and that even imperfect repair delays the onset of proteinuria and end-stage renal failure (Lin *et al.* 2013). The authors concluded that their results 'validated the pursuit of therapeutic approaches aimed at normalising the GBM to prolong kidney function' (Lin *et al.* 2013).

What strategies might these treatments target? Potential strategies are described in Table 1. It is becoming apparent that it will be important to know the underlying genetic mutation before starting treatment in Alport syndrome. Mutations that result in early onset disease warrant more aggressive treatment. The response to treatment is likely to be different even in individuals with different mutations for reasons we do not understand. It is unlikely that any of these treatments will have a uniform benefit in all patients. The benefit of any treatment must always be weighed up against the risks. It will be important to start treatment early, even before the onset of microalbuminuria, as we have seen with ACE inhibitor treatment. None of these treatments is likely to be completely effective and combinations are likely.

Conclusions

There are thus three approaches for the treatment of Alport syndrome based on our understanding of its pathogenesis: firstly, to correct the genetic mutation, for example, by replacing mutant podocytes with cells that produce the normal collagen IV $\alpha 3\alpha 4\alpha 5$ network; secondly, to prevent the development of the abnormal GBM and secondary glomerulosclerosis; and thirdly, to attenuate the compensatory glomerular and tubulointerstitial inflammation (Lin *et al.* 2013).

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Additional information

Competing interests

None declared.

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