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Glomerular pathology in Alport syndrome: a molecular perspective

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Abstract

We have known for some time that mutations in the genes encoding 3 of the 6 type IV collagen chains are the underlying defect responsible for both X-linked (where the COL4A5 gene is involved) and autosomal (where either COL4A3 or COL4A4 genes are involved) Alport syndrome. The result of these mutations is the absence of the sub-epithelial network of all three chains in the glomerular basement membrane (GBM) resulting, at maturity, in a type IV collagen GBM network comprised of only $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains. The altered GBM functions adequately in early life. Eventually there is onset of proteinuria associated with the classic and progressive irregular thickening, thinning, and splitting of the GBM, which culminates in end stage renal failure. We have learned much about the molecular events associated with disease onset and progression through the study of animal models for Alport syndrome, and have identified some potential therapeutic approaches that may serve to delay the onset or slow the progression of the disease. This review focuses on where we are in our understanding of the disease, where we need to go to understand the molecular triggers that set the process in motion, and what emergent therapeutic approaches show promise for ameliorating disease progression in the clinic.

Keywords

Alport Syndrome; glomerular basement membrane; podocyte; pathobiology

Introduction

First described in a British family in 1927 by Dr. Cecil A. Alport, Alport syndrome has become a classic model for diseases of the basement membrane. It was first characterized as a glomerular basement membrane disorder through ultrastructural studies published in 1972 by Spear and Slusser (1). To this day, the characteristic ultrastructural lesions in the GBM remain a critical diagnostic test for the disease, although less invasive and more sophisticated tests are becoming main stream as of late. It wasn't until 1990 when genetic lesions underlying the X-linked form of the disease were identified in the COL4A5 gene that it became apparent that glomerular disease associated with Alport syndrome was caused by defects in the type IV collagen network (2). Due to the obligatory association of $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, and $\alpha 5(\text{IV})$ chains to form the heterotrimeric protomer building blocks that comprise this network, mutations in the COL4A3 or COL4A4 chains were later identified as underlying the autosomal recessive forms of the disease(3,4). The net result is a GBM type IV collagen network comprised entirely of $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains. Interestingly, this alteration in GBM type IV collagen composition does not appear to substantially influence

glomerular function in the early years of life. At some point in early adolescence however, onset of proteinuria occurs, indicating early stages of glomerular disease which invariably progresses to end stage renal failure. The mystery therefore lies in how the change in GBM type IV collagen composition elicits the signals for disease onset and why these cues are delayed until early adolescence. If we can understand the molecular nature of these signals, we may be able to design therapeutic approaches that target them in a way to re-establish the pre-pathogenic state, or suppress the onset of glomerular pathogenesis. This review provides an update of our current understanding of the molecular mechanisms driving Alport glomerular pathogenesis and where we are with regard to emerging therapies. It is clear that re-establishing the normal GBM composition in Alport patients would likely provide a cure for the disease. This could be accomplished through gene replacement or stem cell therapies. Progress using these two approaches in animal models for Alport syndrome is discussed. Lastly, as emergent therapies become apparent, it is imperative to identify the patient population that can benefit from these developments. This will require reliable non-invasive and inexpensive diagnostic techniques as well as the establishment of a global registry for the dissemination of information important to the patient population regarding available clinical trials as well as data from ongoing trials.

Progress in understanding the molecular mechanisms of glomerular pathology

The discovery that Alport syndrome was caused by mutations in type IV collagen genes lead to early speculation regarding how the alteration in basement membrane collagen composition caused the classic GBM ultrastructural dysmorphologies and podocyte foot process effacement associated with glomerular disease progression. The irregular thickening, thinning, and splitting suggested that the GBM might be undergoing proteolytic degradation. Biochemical studies comparing the structure of basement membrane networks demonstrated that those comprised of type IV collagen $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ contained fewer interchain disulfide crosslinks compared to those comprised of type IV collagen $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, and $\alpha 5(\text{IV})$ chains (5). It was surmised that the latter network might therefore be more resistant to endoproteolytic cleavage by glomerular MMPs compared to the former one (5). It wasn't until recently, however, that direct evidence for this relative resistance emerged in the literature (6). In this same study, and several related ones, it was demonstrated that glomeruli in Alport mice show elevated expression of MMPs, including MMP-2, MMP-3, MMP-9 (6), and in a separate study, MMP-12 (7). Treatment of these mice, before the onset of proteinuria, with broadly active inhibitors of MMPs ameliorated Alport renal disease progression, protected against GBM ultrastructural dysmorphology, and extended lifespan, suggesting that the induction of MMPs in these mice plays a direct role in glomerular disease progression, likely via proteolytic degradation of the basement membrane networks. The molecular mechanism underlying induction of the MMPs in Alport glomeruli remains unclear; however recent data suggests a link between biomechanical strain and elevated expression of MMP-3, MMP-9, and MMP-10 in both cultured podocytes and in glomeruli from hypertensive versus normotensive Alport mice (8). Such strain on the capillary tuft may be due to the elevated elasticity of the thinner and less crosslinked type IV collagen network in Alport GBM.

A peculiar aspect of Alport GBM disease is the progressive accumulation of abnormal laminins, including laminin 111 and 211 (9,10). Laminin 111 is expressed during glomerular development and is transcriptionally inactivated in glomerular podocytes upon glomerular maturation in the mouse, at the time when laminin 521 expression, the laminin found in mature GBM, is activated (11). The inactivation occurs normally in Alport glomeruli, but expression is re-activated in mature podocytes resulting in accumulation of laminin 111 in the GBM (12). It is possible that expression of the laminin $\alpha 2$ chain is also activated at this time; however evidence for this event has not been reported. Laminin 111 accumulates

preferentially in the regions of the GBM that are focally thickened, and these regions are more permeable to injected ferritin than other parts of the GBM, suggesting that the accumulation of laminins leads to permeability defects, contributing to the onset and progression of proteinuria (13). Importantly, the accumulation of these abnormal laminins are one of the earliest documented molecular anomalies observed in Alport GBM (12), suggesting that this may constitute a key event in Alport GBM disease initiation. Integrin $\alpha 3$ knockout mice, podocyte specific conditional knockout mice for $\beta 1$ integrin, and CD151 knockout mice have been shown to accumulate laminin $\beta 1$ in the GBM (14-16), suggesting that the origin of laminins in Alport GBM may have something to do with aberrant $\alpha 3\beta 1$ integrin signaling in podocytes. In all three of these mice, ultrastructural changes in the GBM are present that are strikingly similar to that observed in Alport mice. Interestingly, hypertensive Alport mice show accelerated accumulation of laminin 211 in the GBM, suggesting that mechanical stresses on the adhesive interfaces between the glomerular cells and the GBM of the capillary tuft, which are largely mediated through laminin 521/integrin $\alpha 3\beta 1$ adhesive interactions, promotes accumulation of abnormal laminins in Alport GBM.

Disease progression in Alport mice clearly involves cross-talk between glomerular cells. In the case of MMP-12 induction it has been shown that MCP-1 secreted by glomerular mesangial cells acts on the CCR2 receptor on glomerular podocytes resulting in the induction of MMP-12 in glomerular podocytes (7). Inhibition of the CCR2 receptor with the co-receptor inhibitor propagermanium abolishes MMP-12 induction and partially ameliorates the GBM abnormalities and foot process effacement (7). More recently, it has been shown that the bone morphogenetic protein (BMP) antagonist uterine sensitization-associated gene-1 (USAG-1), which is produced by macula densa cells, a cell type in direct contact with glomerular mesangial cells, induces MMP-12 in mesangial cells. Inhibition of USAG-1 by genetic ablation partially ameliorated GBM destruction, inhibited progression of the disease, and extended lifespan (17). Genetic ablation of integrin $\alpha 1$ in Alport mice has a profound effect on disease progression, nearly doubling the lifespan of the animals (9). $\alpha 1\beta 1$ integrin is expressed on glomerular mesangial cells. Its deletion has pleiotropic effects on MMP expression in Alport glomeruli, resulting in marked decreased expression of MMP-12, and elevated expression of gelatinases MMP-2, MMP-9, and MMP-14 (18). In a related study it was shown that inhibition of gelatinase activity in these double knockout mice further protects against GBM destruction (9), demonstrating that MMP dysregulation occurs in both mesangial cells and podocytes, and that secretion of these proteases by both cell types contribute to the GBM destruction associated with the disease.

TGF- $\beta 1$ has been shown to be induced in Alport glomerular podocytes (19), and blocking TGF- $\beta 1$ with a soluble receptor inhibitor reduced focal accumulation of matrix in the GBM, but did not resolve foot process effacement (9). In this same study it was shown that treatment of integrin $\alpha 1$ -null Alport mice with the TGF- $\beta 1$ antagonist restored GBM architecture, prevented the accumulation of abnormal laminins in the GBM, and markedly reduced proteinuria. This suggests that the influences of $\alpha 1\beta 1$ integrin signaling in mesangial cells and TGF- $\beta 1$ on glomerular disease progression in the Alport mouse are distinct. Thus, multiple glomerular cells as well as multiple pathways influence abnormal laminin accumulation in the GBM of Alport mice. Collectively, the data clearly indicates that multiple glomerular cell types participate in the pathogenic process underlying Alport GBM disease initiation and progression.

An interesting but unresolved clue regarding Alport glomerular pathogenesis is a profound influence of background strain in mouse models. Alport mice on the 129 Sv background approach end stage at 8 to 10 weeks of age, and mice with the same mutation on the C57 BL/6 background reach end stage at 25 to 30 weeks (20). These observations reflect quantitative trait loci that have been localized, however, the gene(s) responsible have not yet been

identified. One plausible explanation put forward was based a strain-dependent isoform switch, resulting in the deposition of collagen $\alpha 5\alpha 6(IV)$ networks in the GBM, which occurs in the B1/6 autosomal Alport background, but not in the 129/Sv background (21). This isoform switch may have a small influence on disease progression (C57 B1/6 X-linked Alport mice live an average of 23 $\pm$ 3 weeks versus 27 $\pm$ 3 weeks for C57 B1/6 autosomal Alport mice), however it cannot account for the large difference in lifespan when comparing either model to the 129 Sv Alport mouse (22). Thus the specific influences of the QTLs and the genes involved have yet to be elucidated. Their discovery may reveal key pathways of Alport glomerular pathology and thus new therapeutic targets.

Treatment of Alport syndrome

Early studies were conducted testing the efficacy of angiotensin converting enzyme (ACE) inhibitors using the Samoyed dog model for XLAS (23). These studies showed considerable promise, reducing proteinuria, delayed the decline in glomerular filtration rate, and significantly extended lifespan. Testing of the ACE inhibitor Ramipril, using an autosomal mouse model for Alport syndrome, provided highly significant nephroprotective effects, more than doubling the lifespan and significantly reducing matrix accumulation and TGF- $\beta 1$ expression levels (24). Importantly, the protective effects of Ramipril therapy was most pronounced when animals were dosed at early stages of the disease, suggesting that such therapy in humans would require early diagnosis and preemptive therapy. Small trials using ACE inhibitors on humans with Alport syndrome gave mixed but promising results with individual variation (25,26). Currently, many Alport patients are treated empirically with ACE inhibitors, however efficacy of these treatments will require good documentation regarding the stage of renal disease when treatment is initiated, and solid longitudinal efficacy/safety data. Nonetheless, as of now, ACE inhibition does appear to be the most promising of the approved interventions for the disease.

Other potential therapeutic targets have been implicated through the study of animal models of Alport syndrome. Treatment with the HMG-CoA-reductase inhibitor, Cervistatin, significantly reduced matrix accumulation and expression of both TGF- $\beta 1$ and connective tissue growth factor (CTGF), and significantly improved renal function and extended life span (27). The effects were not as impressive as Ramipril therapy, however. Cyclosporine treatment showed beneficial effects using the Samoyed dog model for XLAS (28), however the use of this drug in children is unlikely to be attempted given the well documented nephrotoxic side effects of the drug. Another emergent drug target is $\alpha 1$ integrin blockade (9), which is at the preclinical testing stage. $\alpha 1$ integrin blockade combined with TGF- $\beta 1$ blockade proved very effective in the Alport mouse model (9). The development of a combination therapy comprised of two novel drugs is unlikely to be attempted in humans however, due to the high risk of side effects. As mentioned above, approaches involving the use of MMP inhibitors show promise in animal models, however all of the small molecules currently available have been tabled due to serious side effects. Development of a truly effective targeted molecular therapeutic awaits discovery key events underlying the initiation of the glomerular disease pathology.

A very logical approach to the treatment of Alport syndrome is to simply provide the appropriate glomerular cells with a corrected version of the defective type IV collagen gene. This could be done in one of two ways: gene replacement therapy or stem cell therapy. The prospect of a gene therapy approach was considered very early with the development of a renal perfusion method to deliver adenoviral vectors containing the LacZ expression cassette (29). The method proved quite promising, achieving strong expression in 80 % of glomeruli using pigs. A similar adenoviral approach was later used to introduce full length human $\alpha 5(IV)$ cDNA to pig kidneys, where expression of the construct/protein was documented in glomeruli, however the transduction efficiency was not as good as that for LacZ (30). In

order for this type of therapy to be effective, one would need to achieve very high transduction efficiency of glomerular podocytes, which at the present time seems out of reach. Furthermore, gene therapy approaches are fraught with risks that have prevented their use throughout much of the mainstream for the treatment of human diseases.

A stem cell based approach for the treatment of Alport syndrome has also been considered. Early attempts using bone marrow transplantation into irradiated Alport mice showed some promising results, with low level recruitment of bone marrow derived cells and some expression of the type IV collagen $\alpha 3/\alpha 4/\alpha 5$ hetero-trimers in the transplanted mice associated with improved renal function (31,32). In a related study it was shown that irradiation alone improved renal function and lifespan in Alport mice, presumably via reduction of leukocytic infiltration (33). This study threw into question whether the improved renal function documented in the bone marrow transplant studies were simply an artifact of irradiation. A follow up study showed that either bone marrow or whole blood from wild type mice transplanted into Alport mice without radiation improved renal function. In these studies more definitive data was provided regarding the wild type origin of glomerular cells in the in the transplanted Alport mice (34). Expression was low, and the extension in lifespan was only about 15%, however the study did provide proof of concept that the transplanted cells may indeed differentiate into glomerular cell types capable of functional complementation for the defective protein. Thus, while in the very early stages of development, stem cell therapy has properties that suggest it might evolve into a useful therapeutic approach sometime in the future. It should be noted, however, that the risks associated with stem cell based therapies are not well understood, since the development of these approaches are still in their infancy.

Diagnostics

It is well established that the first element of diagnosis for Alport syndrome is family history, urinalysis for albumin and microhematuria, and hearing/vision evaluation. If these first tests warrant concern for Alport syndrome, the gold standard for testing has been renal biopsy and electron microscopy to evaluate the ultrastructure of the GBM (35). While biopsy is relatively safe, there are some risks involved which have promoted the development of molecular testing methodologies. One such test requires a skin biopsy combined with immunohistochemical analysis for the type IV collagen $\alpha 5$ chain. The skin contains a type IV collagen network comprise of $\alpha 5(\text{IV})/\alpha 6(\text{IV})$ hetero-trimers, and thus in the case of XLAS, a positive diagnosis can be made by the absence of the $\alpha 5(\text{IV})$ chain in the basement membranes of the skin. This method is not suitable for diagnosing the autosomal recessive forms of the disease since the $\alpha 3(\text{IV})$ and $\alpha 4(\text{IV})$ chains are not expressed in skin. The method has proven particularly useful for the diagnosis of female carriers of XLAS, who generally present a milder form of the disease. Renal biopsies from these patients can be hard to distinguish from thin basement membrane disease, thus this alternate test may be superior to standard transmission electron microscopy for this group. The chimeric nature of cells in these patients, due to random X chromosome inactivation, will produce a broken segmental rather than linear pattern of collagen $\alpha 5(\text{IV})$ immunostaining, providing a reliable diagnostic indicator (36).

Currently a last resort, due to the cost of the methodology, is genetic testing. The methodology, which involves either direct sequencing or chip array analysis, currently suffers from a low mutation detection rate (>50%) and is not yet widely available. Common point mutations can be tested directly for a more rapid, inexpensive, and highly reliable (99% specificity) alternative, however these types of tests are only suitable for testing carrier status in families for which the specific mutation is already known (37). There is little doubt that the continued development of Next Gen sequencing and computation analysis will

allow for direct sequencing and analysis of the genes with high reliability at a low cost, which is most certainly to evolve into the diagnostic standard for the future.

As is evidenced from the discussion of molecular pathology of Alport glomerular disease, most treatment strategies currently under development work best when therapy is started early (before the onset of proteinuria). This trend predicts the best outcomes for patients will require that they be identified very early. A database has been created by the **Alport Foundation** (<http://www.alportsyndrome.org/>) through the University of Minnesota Department of Pediatric Nephrology designated the *Alport Syndrome Treatments and Outcomes Registry* (ASTOR). The registry is a global collaborative effort aiming to create a comprehensive voluntary patient database enabling families and physicians to communicate regarding the evolution of clinical trials for the treatment of the disease. This resource should prove essential for early identification of treatment groups in the future, facilitating the best possible outcomes.

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References

1. Spear GS, Slusser RJ. Alport's syndrome emphasizing electron microscopic studies of the glomerulus. *Am J Pathol.* 1972; 2:213–24. [PubMed: 4343992]
2. Barker DF, Hostikka SL, Zhou J, Chow LT, Oliphant AR, Gerken SC, Gregory MC, Skolnick MH, Atkin CL, Tryggvason K. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science.* 1990; 4960:1224–7. [PubMed: 2349482]
3. Lemmink HH, Mochizuki T, van den Heuvel LP, Schröder CH, Barrientos A, Monnens LA, van Oost BA, Brunner HG, Reenders ST, Smeets HJ. Mutations in the type IV collagen alpha 3 (COL4A3) gene in autosomal recessive Alport syndrome. *Hum Mol Genet.* 1994; 8:1269–73. [PubMed: 7987301]
4. Mochizuki T, Lemmink HH, Mariyama M, Antignac C, Gubler MC, Pirson Y, Verellen-Dumoulin C, Chan B, Schröder CH, Smeets HJ. Identification of mutations in the alpha 3(IV) and alpha 4(IV) collagen genes in autosomal recessive Alport syndrome. *Nat Genet.* 1994; 1:77–81. [PubMed: 7987396]
5. Gunwar S, Ballester F, Noelken ME, Sado Y, Ninomiya Y, Hudson BG. Glomerular basement membrane identification of a novel disulfide-cross-linked network of alpha3, alpha4, and alpha5 chains of type IV collagen and its implications for the pathogenesis of Alport syndrome. *J Biol Chem.* 1998; 15:8767–75. [PubMed: 9535854]
6. Zeisberg M, Khurana M, Rao VH, Cosgrove D, Rougier JP, Werner MC, Shield CF 3rd, Werb Z, Kalluri R. Stage-specific action of matrix metalloproteinases influences progressive hereditary kidney disease. *PLoS Med.* 2006; 4:e100. [PubMed: 16509766]
7. Rao VH, Meehan DT, Delimont D, Nakajima M, Wada T, Gratton MA, Cosgrove D. Role for macrophage metalloelastase in glomerular basement membrane damage associated with alport syndrome. *Am J Pathol.* 2006; 1:32–46. [PubMed: 16816359]
8. Meehan DT, Delimont D, Cheung L, Zallocchi M, Sansom SC, Holzclaw JD, Rao V, Cosgrove D. Biomechanical strain causes maladaptive gene regulation, contributing to Alport glomerular disease. *Kidney Int.* 2009; 9:968–76. [PubMed: 19710627]
9. Cosgrove D, Rodgers K, Meehan D, Miller C, Bovard K, Gilroy A, Gardner H, Kotlianski V, Gotwals P, Amatuucci A, Kalluri R. Integrin alpha1beta1 and transforming growth factor-beta1 play distinct roles in alport glomerular pathogenesis and serve as dual targets for metabolic therapy. *Am J Pathol.* 2000; 5:1649–59. [PubMed: 11073824]
10. Kashtan CE, Kim Y, Lees GE, Thorner PS, Virtanen I, Miner JH. Abnormal glomerular basement membrane laminins in murine, canine, and human Alport syndrome: aberrant laminin alpha2 deposition is species independent. *J Am Soc Nephrol.* 2001; 2:252–60. [PubMed: 11158215]

11. Miner JH, Patton BL, Lentz SI, Gilbert DJ, Snider WD, Jenkins NA, Copeland NG, Sanes JR. The laminin alpha chains: expression, developmental transitions, and chromosomal locations of alpha1-5, identification of heterotrimeric laminins 8-11, and cloning of a novel alpha3 isoform. *J Cell Biol.* 1997; 3:685–701. [PubMed: 9151674]
12. Abrahamson DR, Prettyman AC, Robert B, St John PL. Laminin-1 reexpression in Alport mouse glomerular basement membranes. *Kidney Int.* 2003; 3:826–34. [PubMed: 12631063]
13. Abrahamson DR, Isom K, Roach E, Stroganova L, Zelenchuk A, Miner JH, St John PL. Laminin compensation in collagen alpha3(IV) knockout (Alport) glomeruli contributes to permeability defects. *J Am Soc Nephrol.* 2007; 9:2465–72. [PubMed: 17699809]
14. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R. Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development.* 1996; 11:3537–47. [PubMed: 8951069]
15. Kanasaki K, Kanda Y, Palmsten K, Tanjore H, Lee SB, Lebleu VS, Gattone VH Jr, Kalluri R. Integrin beta1-mediated matrix assembly and signaling are critical for the normal development and function of the kidney glomerulus. *Dev Biol.* 2008; 2:584–93. [PubMed: 18082680]
16. Baleato RM, Guthrie PL, Gubler MC, Ashman LK, Roselli S. Deletion of CD151 results in a strain-dependent glomerular disease due to severe alterations of the glomerular basement membrane. *Am J Pathol.* 2008; 4:927–37. [PubMed: 18787104]
17. Tanaka M, Asada M, Higashi AY, Nakamura J, Oguchi A, Tomita M, Yamada S, Asada N, Takase M, Okuda T, Kawachi H, Economides AN, Robertson E, Takahashi S, Goldschmeding R, Muso E, Fukatsu A, Kita T, Yanagita M. Loss of the BMP antagonist USAG-1 ameliorates disease in a mouse model of the progressive hereditary kidney disease Alport syndrome. *J. Clin. Invest.* 2010; 120:768–77. [PubMed: 20197625]
18. Cosgrove D, Meehan DT, Delimont D, Pozzi A, Chen X, Rodgers KD, Tempero RM, Zallocchi M, Rao VH. Integrin alpha1beta1 regulates matrix metalloproteinases via P38 mitogen-activated protein kinase in mesangial cells: implications for Alport syndrome. *Am J Pathol.* 2008; 3:761–73. [PubMed: 18258846]
19. Sayers R, Kalluri R, Rodgers KD, Shield CF, Meehan DT, Cosgrove D. Role for transforming growth factor-beta1 in alport renal disease progression. *Kidney Int.* 1999; 5:1662–73. [PubMed: 10571774]
20. Andrews KL, Mudd JL, Li C, Miner J. Quantitative trait loci influence renal disease progression in a mouse model of Alport syndrome. *Am. J. Pathol.* 2002; 160:721–30. [PubMed: 11839593]
21. Kang JS, Wang X-P, Miner JH, Morello R, Sado Y, Abrahamson D, Borza D-B. Loss of $\alpha 3\alpha 4$ (IV) collagen from the glomerular basement membrane induces a strain-dependent isoforms switch to $\alpha 5\alpha 6$ (IV) collagen associated with longer renal survival in *Col4a3*^{-/-} Alport mice. *J. Am. Soc. Nephrol.* 2006; 17:1962–69. [PubMed: 16769745]
22. Rheault MN, Kren SM, Theilen BK, Mesa HA, Crosson JT, Thomas W, Sado Y, Kashtan CE, Segal Y. Mouse model for X-linked Alport syndrome. *J. Am. Soc. Nephrol.* 2004; 15:1466–74. [PubMed: 15153557]
23. Grodecki KM, Gains MJ, Bauml R, Osmond DH, Cotter B, Valli VE, Jacobs RM. Treatment of X-linked hereditary nephritis in Samoyed dogs with angiotensin converting enzyme (ACE) inhibitor. *J Comp Pathol.* 1997; 3:209–25. [PubMed: 9447482]
24. Gross O, Beirowski B, Koepke ML, Kuck J, Reiner M, Addicks K, Smyth N, Schulze-Lohoff E, Weber M. Preemptive ramipril therapy delays renal failure and reduces renal fibrosis in COL4A3-knockout mice with Alport syndrome. *Kidney Int.* 2003; 2:438–46. [PubMed: 12631109]
25. Proesmans W, Van Dyck M. Enalapril in children with Alport syndrome. *Pediatr Nephrol.* 2004; 3:271–5. [PubMed: 14745635]
26. Li JG, Ding J, Wang F, Zhang HW. Drugs controlling proteinuria of patients with Alport syndrome. *World J Pediatr.* 2009; 4:308–11. [PubMed: 19911149]
27. Koepke ML, Weber M, Schulze-Lohoff E, Beirowski B, Segerer S, Gross O. Nephroprotective effect of the HMG-CoA-reductase inhibitor cerivastatin in a mouse model of progressive renal fibrosis in Alport syndrome. *Nephrol Dial Transplant.* 2007; 4:1062–9. [PubMed: 17287218]

28. Chen D, Jefferson B, Harvey SJ, Zheng K, Gartley CJ, Jacobs RM, Thorner PS. Cyclosporine a slows the progressive renal disease of alport syndrome (X-linked hereditary nephritis): results from a canine model. *J Am Soc Nephrol.* 2003; 3:690–8. [PubMed: 12595505]
29. Parpala-Spärman T, Lukkarinen O, Heikkilä P, Tryggvason K. A novel surgical organ perfusion method for effective ex vivo and in vivo gene transfer into renal glomerular cells. *Urol Res.* 1999; 2:97–102.
30. Heikkilä P, Tibell A, Morita T, Chen Y, Wu G, Sado Y, Ninomiya Y, Pettersson E, Tryggvason K. Adenovirus-mediated transfer of type IV collagen alpha5 chain cDNA into swine kidney in vivo: deposition of the protein into the glomerular basement membrane. *Gene Ther.* 2001; 11:882–90.
31. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R. Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. *Proc Natl Acad Sci USA.* 2006; 19:7321–6. [PubMed: 16648256]
32. Prodromidi EI, Poulsom R, Jeffery R, Roufosse CA, Pollard PJ, Pusey CD, Cook HT. Bone marrow-derived cells contribute to podocyte regeneration and amelioration of renal disease in a mouse model of Alport syndrome. *Stem Cells.* 2006; 11:2448–55. [PubMed: 16873763]
33. Katayama K, Kawano M, Naito I, Ishikawa H, Sado Y, Asakawa N, Murata T, Oosugi K, Kiyohara M, Ishikawa E, Ito M, Nomura S. Irradiation prolongs survival of Alport mice. *J Am Soc Nephrol.* 2008; 9:1692–700. [PubMed: 18480315]
34. LeBleu V, Sugimoto H, Mundel TM, Gerami-Naini B, Finan E, Miller CA, Gattone VH 2nd, Lu L, Shield CF 3rd, Folkman J, Kalluri R. Stem cell therapies benefit Alport syndrome. *J Am Soc Nephrol.* 2009; 11:2359–70. [PubMed: 19833902]
35. Mazzucco G, De Marchi M, Monga G. Renal biopsy interpretation in Alport Syndrome. *Semin Diagn Pathol.* 2002; 3:133–45. [PubMed: 12180634]
36. Kashtan CE. Alport syndrome and the X chromosome: implications of a diagnosis of Alport syndrome in females. *Nephrol Dial Transplant.* 2007; 6:1499–505. [PubMed: 17400558]
37. Pont-Kingdon G, Sumner K, Gedge F, Miller C, Denison J, Gregory M, Lyon E. Molecular testing for adult type Alport syndrome. *BMC Nephrol.* 2009; 10:38. [PubMed: 19919694]