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REVIEW

Searching for a treatment for Alport syndrome using mouse models

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Abstract

Alport syndrome (AS) is a hereditary nephritis caused by mutations in COL4A3, COL4A4 or COL4A5 encoding the type IV collagen α3, α4, and α5 chains, which are major components of the glomerular basement membrane. About 20 years have passed since COL4A3, COL4A4, and COL4A5 were identified and the first Alport mouse model was developed using a knockout approach. The phenotype of Alport mice is similar to that of Alport patients, including characteristic thickening and splitting of the glomerular basement membrane. Alport mice have been widely used to study the pathogenesis of AS and to develop effective therapies. In this review, the newer therapies for AS, such as pharmacological interventions, genetic approaches and stem cell therapies, are discussed. Although some stem cell therapies have been demonstrated to slow the renal disease progression in Alport mice, these therapies demand continual refinement as research advances. In terms of the pharmacological drugs, angiotensin-converting enzyme inhibitors have been shown to be effective in Alport mice. Novel therapies that can provide a better outcome or lead to a cure are still awaited.

Key words: Alport syndrome; Angiotensin-converting enzyme; Genetic; Hereditary nephritis; Pharmacological; Renal injury; Stem cell therapy

Core tip: There is currently no curative treatment for Alport syndrome, a progressive hereditary nephritis. However, many drugs have been demonstrated to slow the progression of renal injury in Alport mouse models. Alport mice treated with vasopeptidase inhibitors or angiotensin-converting enzyme inhibitors showed a more than two-fold longer survival than untreated Alport mice. A human clinical trial of an angiotensin-converting enzyme inhibitor is currently in progress. Genetic approaches have been used to elucidate the pathogenesis of this progressive renal disease. Stem cell therapies were also attempted, with some beneficial effects; however, they need to be improved before being tested in clinical trials.

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INTRODUCTION

Alport syndrome (AS) is characterized by a classic triad of renal injury, sensorineural deafness and ocular abnormalities[1]. The disease frequency of AS is about 1:5000[2]. AS begins with asymptomatic microscopic hematuria, progresses to characteristic thinning, thickening and splitting of the glomerular basement membrane (GBM), and finally leads to end-stage renal failure[3]. The causative genes of this syndrome are COL4A3, COL4A4 and COL4A5, which are associated with two types of

disease: X-linked and autosomal. The X-linked type of AS is caused by mutations in COL4A5[4], while the autosomal type of AS is caused by mutations in COL4A3 or COL4A4[5,6]. COL4A3, COL4A4 and COL4A5 encode the type Ⅳ collagen α3, α4 and α5 chains, respectively. Since the type Ⅳ collagen α3, α4 and α5 chains are major structural components of the GBM, AS is a type Ⅳ collagen disease.

The purpose of this review is to summarize the current knowledge that has been obtained using mouse models of Alport syndrome.

PATHOGENESIS

At the molecular level, there are only three triple-helical protomers, α1.α1.α2, α3.α4.α5 and α5.α5.α6, in type Ⅳ collagens[7]. The non-collagenous domain (NC1) at the carboxyl terminus of these protomers joins them to each other to make the suprastructure of the GBM. The α1/α1/α2, α1/α2/α5/α6 and α3/α4/α5 heterohexamers were identified by digesting the NC1 hexamer from human glomeruli with bacterial collagenase[7]. Interestingly, the α3/α4/α5 heterohexamer consists of one α4-α4 homodimer and two α3-α5 heterodimers, while the α1/α1/α2 heterohexamer consists of two α1-α1 homodimers and one α2-α2 homodimer, and the α1/α2/α5/α6 heterohexamer consists of two α1-α5 heterodimers and one α2-α6 heterodimer[7]. The α3 (Ⅳ) and α4 (Ⅳ) chains have to accompany the α5 (Ⅳ) chain, and the α3/α4/α5 heterohexamer consists of compositions of $(\alpha3)_2(\alpha4)_2(\alpha5)_2$[7]. NC1 domains were also demonstrated to contain recognition sequences to form α1.α1.α2 (Ⅳ) and α3.α4.α5 (Ⅳ) networks[8].

There is a developmental switch from α1 and α2 (Ⅳ) chains to α3, α4 and α5 (Ⅳ) chains; the GBM from capillary loop stage contains α3, α4 and α5 (Ⅳ) chains, as well as α1 and α2 (Ⅳ) chains, while the GBM at the comma- and S-shaped stages contains only α1 and α2 (Ⅳ) chains[9,10]. In mature glomeruli, the GBM is mainly composed of α3, α4, and α5 (Ⅳ) chains. While only the distal tubular basement membranes (TBMs) were positive for the α3, α4 and α5 (Ⅳ) chains in humans, nearly the full range of TBMs in the mouse are positive for the α3, α4 and α5 (Ⅳ) chains[9].

GBM in X-linked AS patients consists of only α1 and α2 (Ⅳ) chains because the developmental switch does not occur[10]. The loss of the α5 (Ⅳ) chain leads to the loss of all three chains (α3, α4 and α5 (Ⅳ) chains) in the GBM because of the defective assembly of triple-helical α3.α4.α5 (Ⅳ) protomers[11]. This abnormal GBM in X-linked AS patients is more susceptible to proteolysis by bacterial collagenase, cathepsin B, cathepsin G and *Pseudomonas* elastase than that in normal humans[10], because the collagenous domain of α1.α1.α2 (Ⅳ) protomers contains fewer disulfide cross-links than do α3.α4.α5 (Ⅳ) protomers[11].

Interestingly, AS patients with 5' glycine mutations have a later onset of end-stage renal failure than those with 3' glycine mutations, which is compatible with the fact that type Ⅳ collagen assembly starts from the NC1 domain at the carboxyl terminus[12,13].

By generating two hybrid kidneys that contained wild endothelial cells and COL4A3 -/- podocytes or COL4A3 -/- endothelial cells and wild podocytes, type Ⅳ collagen α3, α4 and α5 chains proved to be originally produced specifically by podocytes in the kidney[14], thus suggesting that AS is podocyte-associated disease.

Table 1 Mouse models of Alport syndrome

Gene	Mutation	Ref.
ARAS		
COL4A3	exon 48	[15]
COL4A3	exon 48-50	[16]
COL4A3-COL4A4	COL4A3 exon 2-COL4A4 exon 12	[17]
COL4A4	exon 30	[18]
XLAS		
COL4A5	exon 1	[19]

ARAS: Autosomal recessive Alport syndrome; XLAS: X-linked Alport syndrome.

MOUSE MODELS OF ALPORT SYNDROME

There were two COL4A3 knockout models reported in 1996. One model was generated by cloning a neomycin cassette into exon 48 of COL4A3[15]. The other model was generated by deleting three exons between exons 48 and 50 of COL4A3[16]. Both models aimed to disrupt exons in the NC1 domain, and the resulting phenotypes resembled those of autosomal recessive AS in human. The COL4DELTA3-4 model, which has a large deletion between exon 2 of COL4A3 and exon 12 of COL4A4, was also reported[17]. This mouse model was found because of the observation that there was unexpected renal disease in a transgenic line, and this model had a more severe type of AS than the above COL4A3 knockout models, because the expression of COL4A3 and COL4A4 mRNAs were not detected due to a lack of the intergene region of COL4A3-COL4A4. A new COL4A4 mouse model, which has a splice site mutation and skips exon 30 of Col4a4, was also recently reported[18]. Since this mutation does not cause a frame shift, this mouse model retains a mutant α4 (Ⅳ) chain in the GBM and represents a good new AS model.

Regarding the X-linked type, a COL4A5 knockout model was generated by making a nonsense mutation in exon 1 of COL4A5, and this has made the analysis of female carriers easier[19]. These five mouse models are summarized in Table 1.

The COL4A3 -/- mice have been the most commonly used as a mouse model of AS in experimental studies. This is partly because the survival of COL4A3 -/- mice is less variable than that of COL4A5 -/- mice[15,16,19]. Interestingly, the survival of COL4A3 -/- mice is influenced by the genetic background; being 66 d on a 129X1/SvJ background compared to 194 d on a

Table 2 The efficacy of pharmacological drugs in COL4A3 -/- mice

Drug	Survival (d)	Efficacy
Vasopeptidase inhibitor[22]	172	(+++)
ACE inhibitor[23]	150	(+++)
ARB[24]	98	(++)
HMG-CoA reductase inhibitor[26]	91	(++)
CCR1 inhibitor[27]	86	(+)
TNF-alpha antagonist[29]	81	(+)
Renin inhibitor[30]	78	(+)
Vitamin D analog[31]	75	(+)
Untreated (129SvJ background)	71	

ACE: Angiotensin converting enzyme; ARB: Angiotensin-II receptor blocker; CCR1: Chemokine (CC motif) receptor 1; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; TNF: Tumor necrosis factor.

C57BL/6J background[20]. A linkage analysis of quantitative trait loci identified three markers on chromosome 9 and one marker on chromosome 16 that were suggested to be modifier genes. In this regard, it is important to use appropriate control littermates for all experiments. Although the 129 genetic background is good enough to assess the efficacy of new therapies in AS, the C57 genetic background might be better for assessing the long-term effects of new therapies.

The big difference between COL4A3 -/- mice and COL4A5 -/- mice is the existence of the α5 (IV) chain in the GBM of COL4A3 -/- mice[21]. Of note, the expression level of the α5 (IV) chain is more prominent in mice with a C57 genetic background than in those with a 129 genetic background[21]. To assess the efficacy of regeneration therapy in COL4A3 -/- mice, it is recommended that the α3 and α4 (IV) chains, not the α5 (IV) chain, should be used.

PHARMACOLOGICAL INTERVENTIONS

A vasopeptidase inhibitor, AVE7688, extended the lifespan of COL4A3 -/- mice dramatically, and it is the most effective drug against COL4A3 -/- mice identified so far[22]. The various drugs that have shown efficacy in treating COL4A3 -/- mice are summarized in Table 2.

An angiotensin-converting enzyme (ACE) inhibitor, Ramipril, was demonstrated to be effective for treating COL4A3 -/- mice[23]. Notably, early initiation of ACE inhibitor treatment was associated with a longer survival time, and this indicated that the ACE inhibitor had a renoprotective effect in the COL4A3 -/- mice, regardless of its impact on the blood pressure.

Moreover, Gross *et al*[24] compared the antifibrotic effects between an ACE inhibitor and an angiotensin receptor blocker (ARB), which was also known to be an angiotensin receptor 1 antagonist. Although both drugs prolonged the survival of COL4A3 -/- mice, the ACE inhibitor was much more effective than the ARB. Treatment with an ACE inhibitor reduced the transforming growth factor-beta 1 (TGF-β1) and connective tissue growth factor (CTGF) levels more effectively than did treatment with an ARB, which might explain the different effects between ACE inhibitors and ARBs, because TGF-β1 was demonstrated to be associated with renal disease progression in COL4A3 -/- mice[25].

A 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase inhibitor, which was originally used for the treatment of hypercholesterolemia, showed an antifibrotic effect in COL4A3 -/- mice, because it prolonged the survival by inhibiting the activation of fibrotic markers[26]. Interestingly, late initiation of treatment with the HMG-CoA reductase inhibitor at week 7 prolonged the survival of the mice from 71.3 to 90.5 d, while late initiation of ACE inhibitor treatment did not[23].

A chemokine receptor 1 antagonist, BX471, prolonged the survival of COL4A3 -/- mice by preventing interstitial macrophage recruitment[27]. That study showed the involvement of chemokines in the renal fibrosis of COL4A3 -/- mice. However, Ccl2 blockade did not prolong the survival of COL4A3 -/- mice even though it reduced the number of renal macrophages[28].

A tumor necrosis factor alpha antagonist prolonged the survival of COL4A3 -/- mice by decreasing podocyte apoptosis[29]. Aliskiren, a direct renin inhibitor, prolonged the survival of COL4A3 -/- mice by 18% by downregulating both TGF-β1 and CTGF in the kidney[30]. The combination of paricalcitol with an ACE inhibitor led to longer survival than the combination of calcitriol with the ACE inhibitor, which indicated that the different analogs of the active form of vitamin D exert different effects[31].

A matrix metalloproteinase (MMP) -2, -3, and -9 inhibitor cocktail prolonged the survival of COL4A3 -/- mice if it was administered before the onset of proteinuria[32]. In contrast, late administration of the inhibitor cocktail after the onset of proteinuria aggravated the renal disease of COL4A3 -/- mice, which was associated with increased interstitial fibrosis. This dual effect might explain why MMPs played a pathogenic role in the early stage, although they played a protective role in the late stage of disease in COL4A3 -/- mice[32]. MMP-12, also known as macrophage metalloelastase, was upregulated in the podocytes of Alport mice, and a MMP inhibitor, MMI270, which blocks MMP-2, -3, -9, -12 and -14, prolonged the survival of COL4A3 -/- mice from eight to 10 wk, while treatment with a MMP inhibitor that blocked MMP-2, -3 and -9 did not[33]. The authors of that study also showed that a CC chemokine receptor 2 antagonist, propagermanium, also prolonged the survival of COL4A3 -/- mice from eight to 11 wk.

At present, an ACE inhibitor has been reported to be the most effective treatment in humans[34]. A vasopeptidase inhibitor might be considered as the next candidate, since this drug led to the longest survival in COL4A3 -/- mice (Table 2).

GENETIC APPROACHES

TGF-β1 is involved in the progression of renal disease in COL4A3 -/- mice[25]. TGF-β1 was found to be sig-

nificantly upregulated after the onset of proteinuria. TGF-β1 and integrin α1β1 were found to affect distinct pathways in the pathogenesis of COL4A3 -/- mice[35]. While TGF-β1 inhibition prevented the thickening of the GBM, the deletion of integrin α1β1 diminished the foot process effacement of podocytes. Treatment with a combination of these approaches prolonged the survival of Alport mice. Recently, the same group showed that integrin α1 deletion in COL4A3 -/- mice decreased the mesangial invasion into the capillary loops of glomeruli[36]. Integrin α2 deletion in COL4A3 -/- mice prolonged the survival by 20% on a C57Bl6 background[37].

The deletion of discoidin domain receptor 1 (DDR1) in COL4A3 -/- mice prolonged the survival from 64.3 to 94.2 d[38]. Since DDR1 is expressed in podocytes, these results again showed the importance of podocyte involvement in the pathogenesis of AS.

Uterine sensitization-associated gene-1 (USAG-1) deletion in COL4A3 -/- mice improved the renal phenotype and improved the survival[39]. This result was compatible with the finding that recombinant human bone morphogenetic protein-7 (BMP-7) had a protective effect in COL4A3 -/- mice[40], because USAG-1 is known to counteract BMP-7 and is normally expressed in the distal tubules of the kidney[41]. Interestingly, they found that USAG-1 was also expressed in the macula densa, and showed the possibility of crosstalk between the macula densa and extraglomerular mesangial cells[39].

Although MMPs had been thought to be involved in the damage to the GBM in COL4A3 -/- mice, MMP-9 deletion did not affect the progression of renal disease in these mice[42]. Three MMPs; MMP-2, -3, and -9, were genetically ablated in COL4A3 -/- mice, and compensatory upregulation was shown among these MMPs[32]. Therefore, broad-spectrum MMP inhibition is likely required for any effects associated with the MMPs.

A mouse line which had a yeast artificial chromosome including COL4A3 and COL4A4 was generated, and this transgene could rescue the phenotype of COL4A3 -/- mice[43]. Although the expression level of the COL4A3 and COL4A4 transgenes were about 20% of the levels of COL4A3 and COL4A4 in a wild type mouse, the human α3 and α4 (IV) chains could assemble with the mouse α5 (IV) chain. This finding is very interesting, because the amino acid sequence homology of the α3 and α4 (IV) chains between the human and mouse, which are 79% and 78%, respectively, still allows for the formation of triple-helical α3.α4.α5 (IV) protomers.

The expression of an inducible human/mouse chimeric COL4A3 transgene after birth prolonged the lifespan of COL4A3 -/- mice by expressing α3, α4 and α5 (IV) chains in the GBM[44]. Notably, expression of the inducible transgene after three weeks of age could still rescue the phenotype of COL4A3 -/- mice, and the α3.α4.α5 (IV) protomers could integrate into the damaged GBM that was comprised by mainly a α1.α1.α2 network.

STEM CELL THERAPIES

There have been two reports that showed the efficacy of wild-type bone marrow transplantation (BMT) against the renal injury in COL4A3 -/- mice[45,46]. Prodromidi *et al*[45] reported that the blood urea nitrogen (BUN) and serum creatinine (Cr) levels were significantly improved in COL4A3 -/- mice that received wild-type (WT) bone marrow compared to those that received COL4A3 knockout (KO) mouse bone marrow (Table 3). The renal histopathology showed significant improvement of the glomerular injury and tubulointerstitial fibrosis in the WT to KO transplanted mice than in the KO to KO transplanted mice. Moreover, the α3 (IV) chain could be detected partially by immunofluorescence, but not in a Western blot analysis. Sugimoto *et al*[46] reported similar results (Table 3). They also showed that the BUN, Cr, and renal histopathology were significantly improved in the COL4A3 -/- mice that received 21-wk WT bone marrow than did the mice that received KO mouse bone marrow. An immunofluorescence study showed patchy staining of the α3 (IV) chain in the GBM of WT to KO transplanted mice. These two reports shared a common findings that BMT after irradiation from WT to COL4A3 -/- mice dramatically improved the renal injury even though the expression level of the α3 (IV) chain was very low. Neither group examined the survival after BMT as an absolute evaluation marker, so it is unclear whether the BMT could prolong the survival of the mice.

We also reported the results of BMT after irradiation in COL4A3 -/- mice[47]. In contrast to the previous two reports, the BUN, Cr, renal histopathology and survival were significantly improved in both WT to KO and KO to KO mice compared to the untreated KO mice, but there were no significant differences between the WT to KO and KO to KO mice (Table 3). The de novo expression of the α3 (IV) chain could not be detected in the WT to KO mice by immunofluorescence and Western blot analyses. However, wild type COL4A3 mRNA could be identified in the WT to KO, not in the KO to KO, mice by reverse transcription polymerase chain reaction. In fact, fewer than 1% of the podocytes were donor-derived when BMT was performed in a mouse model of mesangial sclerosis[48]. Since KO bone marrow had similar effects as WT bone marrow in the COL4A3 -/- mice, the effect of irradiation itself was examined at sublethal doses. Surprisingly, a sublethal dose of irradiation without subsequent BMT improved the survival of COL4A3 -/- mice. This suggests that the renal injury of COL4A3 -/- mice was improved by the irradiation, not by the BMT. The mechanism by which irradiation improved the survival remains to be clarified, since radiation exposure induces numerous effects.

Another group reported that multipotent mesenchymal stromal cells (MSCs) could not prolong the survival of COL4A3 -/- mice although they improved the interstitial fibrosis by producing vascular endothelial growth

Table 3 The effects of bone marrow transplantation therapy in COL4A3 -/- mice

	Prodromidi *et al*[45]		Sugimot *et al*[46]		Katayama *et al*[47]	
	WT -> KO	KO -> KO	WT -> KO	KO -> KO	WT -> KO	KO -> KO
α3 (IV) IF	+	-	+	-	-	-
α3 (IV) WB	no data	no data	+	-	-	-
Col4a3 mRNA	+	-	+	-	+	-
BUN and Cr	improved	no change	improved	no change	improved	improved
Renal pathology	improved	no change	improved	no change	improved	improved
Survival (d)	no data	no data	no data	no data	125	135

WT: Wild-type; KO: Knockout; IF: Immunofluorescence; WB: Western blot; BUN: Blood urea nitrogen; Cr: Creatinine.

factor[49]. MSCs in the kidney that transdifferentiated into renal cells could not be identified.

However, wild-type bone marrow cells were also shown to prolong the survival of unirradiated COL4A3 -/- mice[50]. Surprisingly, wild-type blood transfusion, as well as the injection of undifferentiated mouse embryonic stem cells, improved the renal function of unirradiated COL4A3 -/- mice, with the appearance of the *de novo* expression of the α3 (IV) chain in the GBM. Although these data confirmed that cell-based therapies could be effective, there was a large discrepancy between the expression patterns of the α3 and α5 (IV) chains: the expression of the α3 (IV) chain was patchy, while that of the α5 (IV) chain was linear. There might be an unknown association between the small amount of *de novo* α3 (IV) chains and the renal improvement of COL4A3 -/- mice that received WT bone marrow. Of interest, a single injection of amniotic fluid stem cells was recently shown to prolong the survival of COL4A5 -/- mice without *de novo* expression of α5 (IV) chains[51].

CONCLUSION

At present, there is no treatment available that can cure AS, and symptomatic renal protective therapies are currently the mainstay of treatment for AS. During the search for a treatment in Alport mice, ACE inhibitors were found to be the most promising therapeutic drugs as first-line therapy. This is a good example of the benefits of mouse studies, because this has led to a double-blind, randomized, placebo-controlled, multicenter EARLY PRO-TECT Alport trial[52]. BMT therapy is also promising, but is still controversial, given the fact that BMT itself is invasive[53]. Other therapeutic agents that have been proven effective in AS mouse models should be considered as the next options for clinical trials in patients with AS.

REFERENCES

1 **Alport AC**. Hereditary familial congenital haemorrhagic nephritis. *Br Med J* 1927; **1**: 504-506 [PMID: 20773074 DOI: 10.1136/bmj.1.3454.504]

2 **Hasstedt SJ**, Atkin CL. X-linked inheritance of Alport syndrome: family P revisited. *Am J Hum Genet* 1983; **35**: 1241-1251 [PMID: 6650503]

3 **Jais JP**, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, Weber M, Gross O, Netzer KO, Flinter F, Pirson Y, Verellen C, Wieslander J, Persson U, Tryggvason K, Martin P, Hertz JM, Schröder C, Sanak M, Krejcova S, Carvalho MF, Saus J, Antignac C, Smeets H, Gubler MC. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol* 2000; **11**: 649-657 [PMID: 10752524]

4 **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; **248**: 1224-1227 [PMID: 2349482 DOI: 10.1126/science.2349482]

5 **Lemmink HH**, Mochizuki T, van den Heuvel LP, Schröder CH, Barrientos A, Monnens LA, van Oost BA, Brunner HG, Reeders ST, Smeets HJ. Mutations in the type IV collagen alpha 3 (COL4A3) gene in autosomal recessive Alport syndrome. *Hum Mol Genet* 1994; **3**: 1269-1273 [PMID: 7987301 DOI: 10.1093/hmg/3.8.1269]

6 **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; **8**: 77-81 [PMID: 7987396 DOI: 10.1038/ng0994-77]

7 **Borza DB**, Bondar O, Todd P, Sundaramoorthy M, Sado Y, Ninomiya Y, Hudson BG. Quaternary organization of the goodpasture autoantigen, the alpha 3(IV) collagen chain. Sequestration of two cryptic autoepitopes by intrapromoter interactions with the alpha4 and alpha5 NC1 domains. *J Biol Chem* 2002; **277**: 40075-40083 [PMID: 12193605 DOI: 10.1074/jbc.M207769200]

8 **Boutaud A**, Borza DB, Bondar O, Gunwar S, Netzer KO, Singh N, Ninomiya Y, Sado Y, Noelken ME, Hudson BG. Type IV collagen of the glomerular basement membrane. Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. *J Biol Chem* 2000; **275**: 30716-30724 [PMID: 10896941 DOI: 10.1074/jbc.M004569200]

9 **Miner JH**, Sanes JR. Collagen IV alpha 3, alpha 4, and alpha 5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. *J Cell Biol* 1994; **127**: 879-891 [PMID: 7962065 DOI: 10.1083/jcb.127.3.879]

10 **Kalluri R**, Shield CF, Todd P, Hudson BG, Neilson EG. Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. *J Clin Invest* 1997; **99**: 2470-2478 [PMID: 9153291 DOI: 10.1172/JCI119431]

11 **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; **273**: 8767-8775 [PMID: 9535854 DOI: 10.1074/

jbc.273.15.8767]
12 **Gross O**, Netzer KO, Lambrecht R, Seibold S, Weber M. Meta-analysis of genotype-phenotype correlation in X-linked Alport syndrome: impact on clinical counselling. *Nephrol Dial Transplant* 2002; **17**: 1218-1227 [PMID: 12105244 DOI: 10.1093/ndt/17.7.1218]
13 **Dölz R**, Engel J, Kühn K. Folding of collagen IV. *Eur J Biochem* 1988; **178**: 357-366 [PMID: 2850175 DOI: 10.1111/j.1432-1033.1988.tb14458.x]
14 **Abrahamson DR**, Hudson BG, Stroganova L, Borza DB, St John PL. Cellular origins of type IV collagen networks in developing glomeruli. *J Am Soc Nephrol* 2009; **20**: 1471-1479 [PMID: 19423686 DOI: 10.1681/ASN.2008101086.]
15 **Cosgrove D**, Meehan DT, Grunkemeyer JA, Kornak JM, Sayers R, Hunter WJ, Samuelson GC. Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. *Genes Dev* 1996; **10**: 2981-2992 [PMID: 8956999 DOI: 10.1101/gad.10.23.2981]
16 **Miner JH**, Sanes JR. Molecular and functional defects in kidneys of mice lacking collagen alpha 3(IV): implications for Alport syndrome. *J Cell Biol* 1996; **135**: 1403-1413 [PMID: 8947561 DOI: 10.1083/jcb.135.5.1403]
17 **Lu W**, Phillips CL, Killen PD, Hlaing T, Harrison WR, Elder FF, Miner JH, Overbeek PA, Meisler MH. Insertional mutation of the collagen genes Col4a3 and Col4a4 in a mouse model of Alport syndrome. *Genomics* 1999; **61**: 113-124 [PMID: 10534397 DOI: 10.1006/geno.1999.5943]
18 **Korstanje R**, Caputo CR, Doty RA, Cook SA, Bronson RT, Davisson MT, Miner JH. A mouse Col4a4 mutation causing Alport glomerulosclerosis with abnormal collagen α3α4α5(IV) trimers. *Kidney Int* 2014; **85**: 1461-1468 [PMID: 24522496 DOI: 10.1038/ki.2013.493]
19 **Rheault MN**, Kren SM, Thielen BK, Mesa HA, Crosson JT, Thomas W, Sado Y, Kashtan CE, Segal Y. Mouse model of X-linked Alport syndrome. *J Am Soc Nephrol* 2004; **15**: 1466-1474 [PMID: 15153557 DOI: 10.1097/01.ASN.0000130562.90255.8F]
20 **Andrews KL**, Mudd JL, Li C, Miner JH. Quantitative trait loci influence renal disease progression in a mouse model of Alport syndrome. *Am J Pathol* 2002; **160**: 721-730 [PMID: 11839593 DOI: 10.1016/S0002-9440(10)64892-4]
21 **Kang JS**, Wang XP, Miner JH, Morello R, Sado Y, Abrahamson DR, Borza DB. Loss of alpha3/alpha4(IV) collagen from the glomerular basement membrane induces a strain-dependent isoform switch to alpha5alpha6(IV) collagen associated with longer renal survival in Col4a3-/- Alport mice. *J Am Soc Nephrol* 2006; **17**: 1962-1969 [PMID: 16769745 DOI: 10.1681/ASN.2006020165]
22 **Gross O**, Koepke ML, Beirowski B, Schulze-Lohoff E, Segerer S, Weber M. Nephroprotection by antifibrotic and anti-inflammatory effects of the vasopeptidase inhibitor AVE7688. *Kidney Int* 2005; **68**: 456-463 [PMID: 16014022 DOI: 10.1111/j.1523-1755.2005.00423.x]
23 **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; **63**: 438-446 [PMID: 12631109 DOI: 10.1046/j.1523-1755.2003.00779.x]
24 **Gross O**, Schulze-Lohoff E, Koepke ML, Beirowski B, Addicks K, Bloch W, Smyth N, Weber M. Antifibrotic, nephroprotective potential of ACE inhibitor vs AT1 antagonist in a murine model of renal fibrosis. *Nephrol Dial Transplant* 2004; **19**: 1716-1723 [PMID: 15128880 DOI: 10.1093/ndt/gfh219]
25 **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; **56**: 1662-1673 [PMID: 10571774 DOI: 10.1046/j.1523-1755.1999.00744.x]
26 **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; **22**: 1062-1069 [PMID: 17287218 DOI: 10.1093/ndt/gfl810]
27 **Ninichuk V**, Gross O, Reichel C, Khandoga A, Pawar RD, Ciubar R, Segerer S, Belemezova E, Radomska E, Luckow B, Perez de Lema G, Murphy PM, Gao JL, Henger A, Kretzler M, Horuk R, Weber M, Krombach F, Schlöndorff D, Anders HJ. Delayed chemokine receptor 1 blockade prolongs survival in collagen 4A3-deficient mice with Alport disease. *J Am Soc Nephrol* 2005; **16**: 977-985 [PMID: 15716328 DOI: 10.1681/ASN.2004100871]
28 **Clauss S**, Gross O, Kulkarni O, Avila-Ferrufino A, Radomska E, Segerer S, Eulberg D, Klussmann S, Anders HJ. Ccl2/Mcp-1 blockade reduces glomerular and interstitial macrophages but does not ameliorate renal pathology in collagen4A3-deficient mice with autosomal recessive Alport nephropathy. *J Pathol* 2009; **218**: 40-47 [PMID: 19156777 DOI: 10.1002/path.2505]
29 **Ryu M**, Mulay SR, Miosge N, Gross O, Anders HJ. Tumour necrosis factor-α drives Alport glomerulosclerosis in mice by promoting podocyte apoptosis. *J Pathol* 2012; **226**: 120-131 [PMID: 21953121 DOI: 10.1002/path.2979]
30 **Gross O**, Girgert R, Rubel D, Temme J, Theissen S, Müller GA. Renal protective effects of aliskiren beyond its antihypertensive property in a mouse model of progressive fibrosis. *Am J Hypertens* 2011; **24**: 355-361 [PMID: 21127470 DOI: 10.1038/ajh.2010.231]
31 **Rubel D**, Stock J, Ciner A, Hiller H, Girgert R, Müller GA, Gross O. Antifibrotic, nephroprotective effects of paricalcitol versus calcitriol on top of ACE-inhibitor therapy in the COL4A3 knockout mouse model for progressive renal fibrosis. *Nephrol Dial Transplant* 2014; **29**: 1012-1019 [PMID: 24198271 DOI: 10.1093/ndt/gft434]
32 **Zeisberg M**, Khurana M, Rao VH, Cosgrove D, Rougier JP, Werner MC, Shield CF, Werb Z, Kalluri R. Stage-specific action of matrix metalloproteinases influences progressive hereditary kidney disease. *PLoS Med* 2006; **3**: e100 [PMID: 16509766 DOI: 10.1371/journal.pmed.0030100]
33 **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; **169**: 32-46 [PMID: 16816359 DOI: 10.2353/ajpath.2006.050896]
34 **Gross O**, Licht C, Anders HJ, Hoppe B, Beck B, Tönshoff B, Höcker B, Wygoda S, Ehrich JH, Pape L, Konrad M, Rascher W, Dötsch J, Müller-Wiefel DE, Hoyer P, Knebelmann B, Pirson Y, Grunfeld JP, Niaudet P, Cochat P, Heidet L, Lebbah S, Torra R, Friede T, Lange K, Müller GA, Weber M. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int* 2012; **81**: 494-501 [PMID: 22166847 DOI: 10.1038/ki.2011.407]
35 **Cosgrove D**, Rodgers K, Meehan D, Miller C, Bovard K, Gilroy A, Gardner H, Kotelianski V, Gotwals P, Amatucci 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; **157**: 1649-1659 [PMID: 11073824]
36 **Zallocchi M**, Johnson BM, Meehan DT, Delimont D, Cosgrove D. α1β1 integrin/Rac1-dependent mesangial invasion of glomerular capillaries in Alport syndrome. *Am J Pathol* 2013; **183**: 1269-1280 [PMID: 23911822 DOI: 10.1016/j.ajpath.2013.06.015]
37 **Rubel D**, Frese J, Martin M, Leibnitz A, Girgert R, Miosge N, Eckes B, Müller GA, Gross O. Collagen receptors integrin alpha2beta1 and discoidin domain receptor 1 regulate maturation of the glomerular basement membrane and loss of integrin alpha2beta1 delays kidney fibrosis in COL4A3 knockout mice. *Matrix Biol* 2014; **34**: 13-21 [PMID: 24480069 DOI: 10.1016/j.matbio.2014.01.006]

38 **Gross O**, Girgert R, Beirowski B, Kretzler M, Kang HG, Kruegel J, Miosge N, Busse AC, Segerer S, Vogel WF, Müller GA, Weber M. Loss of collagen-receptor DDR1 delays renal fibrosis in hereditary type IV collagen disease. *Matrix Biol* 2010; **29**: 346-356 [PMID: 20307660 DOI: 10.1016/j.matbio.2010.03.002]

39 **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, Sakurai T, 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**: 768-777 [PMID: 20197625 DOI: 10.1172/JCI39569]

40 **Zeisberg M**, Bottiglio C, Kumar N, Maeshima Y, Strutz F, Müller GA, Kalluri R. Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. *Am J Physiol Renal Physiol* 2003; **285**: F1060-F1067 [PMID: 12915382]

41 **Tanaka M**, Endo S, Okuda T, Economides AN, Valenzuela DM, Murphy AJ, Robertson E, Sakurai T, Fukatsu A, Yancopoulos GD, Kita T, Yanagita M. Expression of BMP-7 and USAG-1 (a BMP antagonist) in kidney development and injury. *Kidney Int* 2008; **73**: 181-191 [PMID: 17943079 DOI: 10.1038/sj.ki.5002626]

42 **Andrews KL**, Betsuyaku T, Rogers S, Shipley JM, Senior RM, Miner JH. Gelatinase B (MMP-9) is not essential in the normal kidney and does not influence progression of renal disease in a mouse model of Alport syndrome. *Am J Pathol* 2000; **157**: 303-311 [PMID: 10880400 DOI: 10.1016/S0002-9440(10)64541-5]

43 **Heidet L**, Borza DB, Jouin M, Sich M, Mattei MG, Sado Y, Hudson BG, Hastie N, Antignac C, Gubler MC. A human-mouse chimera of the alpha3alpha4alpha5(IV) collagen protomer rescues the renal phenotype in Col4a3-/- Alport mice. *Am J Pathol* 2003; **163**: 1633-1644 [PMID: 14507670 DOI: 10.1016/S0002-9440(10)63520-1]

44 **Lin X**, Suh JH, Go G, Miner JH. Feasibility of repairing glomerular basement membrane defects in Alport syndrome. *J Am Soc Nephrol* 2014; **25**: 687-692 [PMID: 24262794 DOI: 10.1681/ASN.2013070798]

45 **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; **24**: 2448-2455 [PMID: 16873763 DOI: 10.1634/stemcells.2006-0201]

46 **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; **103**: 7321-7326 [PMID: 16648256 DOI: 10.1073/pnas.0601436103]

47 **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; **19**: 1692-1700 [PMID: 18480315 DOI: 10.1681/ASN.2007070829]

48 **Guo JK**, Schedl A, Krause DS. Bone marrow transplantation can attenuate the progression of mesangial sclerosis. *Stem Cells* 2006; **24**: 406-415 [PMID: 16150922 DOI: 10.1634/stemcells.2005-0139]

49 **Ninichuk V**, Gross O, Segerer S, Hoffmann R, Radomska E, Buchstaller A, Huss R, Akis N, Schlöndorff D, Anders HJ. Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. *Kidney Int* 2006; **70**: 121-129 [PMID: 16723981 DOI: 10.1038/sj.ki.5001521]

50 **LeBleu V**, Sugimoto H, Mundel TM, Gerami-Naini B, Finan E, Miller CA, Gattone VH, Lu L, Shield CF, Folkman J, Kalluri R. Stem cell therapies benefit Alport syndrome. *J Am Soc Nephrol* 2009; **20**: 2359-2370 [PMID: 19833902 DOI: 10.1681/ASN.2009010123]

51 **Sedrakyan S**, Da Sacco S, Milanesi A, Shiri L, Petrosyan A, Varimezova R, Warburton D, Lemley KV, De Filippo RE, Perin L. Injection of amniotic fluid stem cells delays progression of renal fibrosis. *J Am Soc Nephrol* 2012; **23**: 661-673 [PMID: 22302195 DOI: 10.1681/ASN.2011030243]

52 **Gross O**, Friede T, Hilgers R, Görlitz A, Gavénis K, Ahmed R, Dürr U. Safety and Efficacy of the ACE-Inhibitor Ramipril in Alport Syndrome: The Double-Blind, Randomized, Placebo-Controlled, Multicenter Phase III EARLY PRO-TECT Alport Trial in Pediatric Patients. *ISRN Pediatr* 2012; **2012**: 436046 [PMID: 22811928 DOI: 10.5402/2012/436046]

53 **Mojahedi MJ**, Hekmat R, Ahmadnia H. Kidney transplantation in patients with alport syndrome. *Urol J* 2007; **4**: 234-237 [PMID: 18270949]

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