FEATURED NEW INVESTIGATOR

Glomerular basement membrane and related glomerular disease

YING MAGGIE CHEN, and JEFFREY H. MINER

ST. LOUIS, MO

The glomerular basement membrane (GBM) is lined by fenestrated endothelium from the capillary-lumen side and by interdigitating foot processes of the podocytes from the urinary- space side. These three layers of the glomerular capillary wall constitute the functional unit of the glomerular filtration barrier. The GBM is assembled through an interweaving of type IV collagen with laminins, nidogen, and sulfated proteoglycans. Mutations in genes encoding *LAMB2, COL4A3, COL4A4, and COL4A5* cause glomerular disease in humans as well as in mice. In addition, laminin α5 mutation in podocytes leads to proteinuria and renal failure in mice. Moreover, more neoepitopes in Goodpasture's disease and for the first time alloepitopes in Alport post-transplantation nephritis have been located in the collagen α5(IV) NC1 domain. These discoveries underscore the importance of the GBM in establishing and maintaining the integrity of the glomerular filtration barrier. (Translational Research 2012;160:291–297)

Abbreviations: APTN = Alport post-transplantation nephritis; AS = Alport syndrome; BM = basement membrane; CCR2 = CC chemokine receptor 2; EA = epitope A; EB = epitope B; GAG = glycosaminoglycan; GBM = glomerular basement membrane; GFB = glomerular filtration barrier; HSPG = heparan sulfate proteoglycan; LAMB2 = laminin b2; LG domain = laminin globular domain; LN = laminin NH2-terminal; MCP-1 = monocyte chemoattractant protein -1; MM = mesangial matrix; MMP-12 = matrix metalloproteinase-12; NC1 = noncollagenous domain 1; SD = slit diaphragm; TBMN = thin basement membrane nephropathy

The kidney glomerular basement membrane (GBM) is an unusually thick basement membrane (BM) formed *via* fusion of distinct BMs assembled by podocytes and glomerular endothelial cells.[1] BMs are sheets of specialized extracellular matrix that underlie all endothelial and epithelial cells and surround all muscle cells, fat cells, and peripheral nerves. They influence cell proliferation, differentiation, migration, and survival. BMs are also involved in filtration, in tissue compartmentalization, and in maintenance of epithelial

Ying Maggie Chen, MD, PhD, *is an Assistant Professor of Medicine in the Renal Division of the Washington University School of Medicine. Her article is based on a presentation given at the Combined Annual Meeting of the Central Society for Clinical Research and Midwestern Section American Federation for Medical Research held in Chicago, Ill, April 2011.*

From the Renal Division; Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Mo.

Our relevant research was supported by NIH grants T32DK007126, K08DK089015, and P30DK079333 (pilot and feasibility study) to Y.M.C., and R01DK078314, R01GM060432, and R01DK081156 to J.H.M.

Conflict of interest: None.

Submitted for publication October 2, 2011; revision submitted February 20, 2012; accepted for publication March 15, 2012.

Reprint requests: Ying Maggie Chen, Washington University School of Medicine, Renal Division 8126, 660 S. Euclid Ave. St. Louis, MO 63110; e-mail: ychen@dom.wustl.edu.

1931-5244/$ - see front matter

© 2012 Mosby, Inc. All rights reserved.

doi:10.1016/j.trsl.2012.03.004

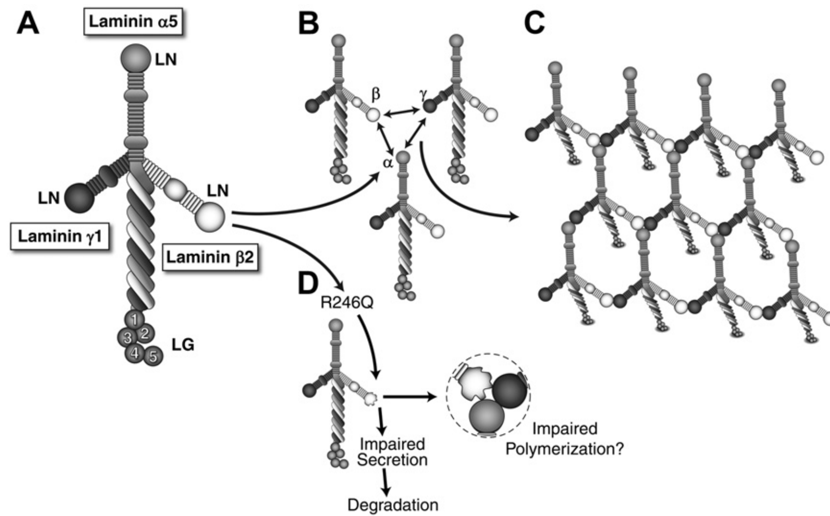

Fig 1. Laminin trimers and assembly. **A**, Structure of a typical cruciform laminin $\alpha\beta\gamma$ heterotrimer, with some domain names indicated. **B** and **C,** Mechanism for polymerization of laminin trimers. **D,** The proposed mechanisms of the R246Q missense mutation causing congenital nephrotic syndrome. It may inhibit laminin secretion from podocytes, eventually leading to degradation intracellularly. In addition, the affected R246Q in the LN domain of laminin β2 may impair the polymerization of laminin trimers to form the GBM. (Permission granted by Journal of the American Society of Nephrology.)

integrity. The GBM, like all BMs, contains members of 4 classes of proteins: laminin, type IV collagen, nidogen, and sulfated proteoglycans.[2]

Of the 9 known matrix proteins present in the mature GBM (laminins α5, β2, γ1; collagen IV α3, α4, α5; nidogen-1 and nidogen-2; and agrin), mutations in 4 of them (laminin β2, collagen α3(IV), collagen α4 [IV], and collagen α5[IV]) have been identified to cause glomerular disease in human. Furthermore, the new identification of collagen α5(IV) noncollagenous domain 1 (NC1) epitopes targeted by Goodpasture autoantibodies or Alport post-transplantation nephritis alloantibodies has provided novel insights into the molecular basis of these 2 forms of anti-GBM nephritis.

LAMININ AND PIERSON SYNDROME

Laminins are heterotrimeric glycoproteins containing 1 α, 1 β, and 1 γ chain. Figure 1, *A* shows a typical laminin heterotrimer. The major laminin heterotrimer in the mature GBM is laminin $\alpha5\beta2\gamma1$, or LM-521.[3] LM-521 is secreted by both podocytes and endothelial cells.[4] During glomerulogenesis, there is a transition in laminin gene expression, such that the $\alpha1\beta1\gamma1$ (LM-111) and $\alpha5\beta1\gamma1$ (LM-511) trimers are present in the nascent GBM, but are replaced by LM-521 as maturation progresses.[5,6] Laminin trimerization occurs in the endoplasmic reticulum and involves association of the three chains along their α-helical laminin coiled-coil domains to form the long arm.[7] Once trimers are secreted into the extracellular space, they polymerize to form a supramolecular network *via* interactions among the α, β, and γ short arm NH2-termini (called LN domains)[8] (Fig 1, *B and C*). The large COOH-terminal laminin globular (LG) domain of α chains mediates laminin and BM interactions with cellular receptors. Therefore, laminin polymerization both initiates basement membrane formation and provides signals to the adjacent cells.[9]

Laminin β2 (LAMB2) is a component of laminin-521. Truncating or severe missense mutations in *LAMB2* cause Pierson syndrome.[10] Also called microcoria-congenital nephrosis syndrome, Pierson syndrome is a rare autosomal recessive disease characterized by congenital nephrotic syndrome/diffuse mesangial sclerosis, distinct ocular abnormalities including microcoria (small pupils), muscular hypotonia, and impairment of vision and neurodevelopment.[11-13] Children affected by Pierson syndrome usually die within days or weeks after birth from renal failure. However, with dialysis and therapeutic nephrectomy, a few have lived for up to 2 years. *Lamb2-/-* mice recapitulate the features of Pierson syndrome.[14-19] In contrast, patients with some less severe missense LAMB2 mutations, such as R246Q and C321R, exhibit nephrotic syndrome with significantly milder extrarenal defects.[20,21]

To begin to investigate how specific missense mutations in *LAMB2* cause proteinuria, we generated 3 lines of transgenic mice, each with differing podocyte expression levels of R246Q-mutant rat laminin β2 (Tg^{Lo}, Tg^{Med}, and Tg^{Hi}). The transgene-derived R246Q-mutant

$\beta 2$ replaced the wild-type mouse laminin $\beta 2$ in the GBM. These transgenic mice developed much less severe proteinuria than their nontransgenic *Lamb2*-deficient littermates; the level of proteinuria correlated inversely with the level of R246Q-LAMB2 expression. *In vitro,* we tried to synthesize NH2-terminal fragments of rat laminin $\beta 2$ containing the R246Q, R246W and C321R mutations (respectively) in transfected HEK 293 cells using a system in which the LN and LEa domains were fused to a human Ig Fc domain. Although the wild-type fusion protein was secreted into the medium, the mutant fusion proteins were not. Analysis of cell lysates showed that the mutant proteins were synthesized, but they remained inside the cells. These biochemical studies demonstrated that the missense mutations result in impaired secretion of laminin. Together with our transgenic mouse studies, our results suggest that the R246Q mutation causes nephrotic syndrome by impairing secretion of laminin-521 from podocytes into the GBM, resulting in subnormal GBM laminin levels, leading to a more porous GBM and a leaky glomerular filtration barrier (GFB). However, high-level expression of the R246Q mutant in Tg^{Hi} mutants mostly overcomes the effects of the secretion defect[22] (Fig 1*D*). Our findings highlight the importance of *LAMB2* mutations in the pathogenesis of congenital nephrotic syndrome and suggest that therapies that can increase either expression or secretion of the mutant form should improve selectivity of the defective GFB.

While no mutations affecting human laminin $\alpha 5$ or laminin $\gamma 1$ have been reported, podocyte-specific ablation of *Lamα5* resulted in varying degrees of proteinuria and progression to renal failure, though the overall renal phenotype was never as severe as observed in *Lamb2*-/- mice.[23] This is likely due to the inefficiency of Cre vs the germ-line deletion of *Lamb2*.

COLLAGEN IV AND HEREDITARY GBM DISEASE -ALPORT SYNDROME AND THIN BASEMENT MEMBRANE DISEASE

There are 6 chains of type IV collagen, $\alpha 1$ to $\alpha 6$, encoded by 3 pairs of genes on chromosomes 2, 13, and X. Each chain has 3 domains: a short 7S domain at the N-terminus, a long, interrupted collagenous domain in the middle, and a noncollagenous domain (NC1) at the C-terminus. The 6 distinct α chains are arranged into 3 different triple helical heterotrimeric protomers: $(\alpha 1)_2\alpha 2$, $\alpha 3\alpha 4\alpha 5$, and $(\alpha 5)_2\alpha 6$. Protomers polymerize to create collagen networks by uniting 2 trimeric NC1 domains to form NC1 hexamers, and by uniting 4 trimeric 7S domains to form 7S dodecamers.[24] At early stages of glomerulogenesis, the $(\alpha 1)_2\alpha 2$ network is a component of GBM, Bowman's capsule and mesangial matrix (MM). During normal glomerulogenesis, most of the $(\alpha 1)_2\alpha 2$ network is replaced by $\alpha 3\alpha 4\alpha 5$ in the GBM and by $(\alpha 5)_2\alpha 6$ in Bowman's capsule, with $(\alpha 1)_2\alpha 2$ remaining in the subendothelial region of the GBM and in the MM 6.[25] Experiments in mice showed that podocytes, but not endothelial cells, synthesize the $\alpha 3\alpha 4\alpha 5$ network.[26]

Alport syndrome (AS) and thin basement membrane nephropathy (TBMN) are genetically heterogenous conditions characterized by structural abnormalities in the GBM. Although both conditions typically present with hematuria, AS is associated with proteinuria, progressive renal failure, and extrarenal syndromes. In contrast, TBMN is characterized by isolated persistent or recurrent hematuria, and generally never progresses toward end stage renal disease. The hall mark of TBMN is diffuse attenuation of the GBM, which also resembles the ultrastructural changes of early AS patients or Alport carriers.

Eighty-five percent of AS patients have the X-linked form due to mutations in *COL4A5*. Although in most X-linked AS patients, the $\alpha 5$ chain of type IV collagen is missing from the GBM, in one study it was shown that the *COL4A5* transcript was clearly expressed in the podocytes of one of the three X-linked AS patients studied. Moreover, *COL4A3* and *COL4A4* transcripts were readily detected in the podocytes of all three patients despite the co-absence of these two chains from the GBM. These data suggest a post-transcriptional regulatory mechanism for type IV collagen chains and that transcription of the three genes is not coregulated.[27] The *COL4A5* mutations also led to strong accumulation of $\alpha 1$ and $\alpha 2$ chains of type IV collagen across the entire width of the GBM, and the absence of the $(\alpha 5)_2\alpha 6$ network in Bowman's capsule. Concomitant mutations in the $\alpha 6$ gene, which is tightly lined to $\alpha 5$ on the X, are associated with diffuse leiomyomatosis, although the mechanism of pathogenesis remains unclear.

The *COL4A3/4* genes are involved in both autosomal AS and TBMN. Autosomal recessive AS accounts for about 15% of affected individuals and arises from either compound heterozygous or homozygous mutations in *COL4A3* or *COL4A4*. Autosomal dominant AS and 40% of TBMN are caused by heterozygous mutations in *COL4A3* or *COL4A4*. Heterozygous mutations in *COL4A3* or *COL4A4* typically cause the mild abnormalities seen in TBMN, although in rare cases, heterozygous mutations are associated with autosomal dominant AS.[28] However, although approximately 40% of patients with TBMN can be considered carriers for autosomal recessive AS,[29] linkage to *COL4A3* and *COL4A4* has been excluded in some families with TBMD, indicating that TBMD is a genetically heterogeneous condition.[30]

The collagen $\alpha3\alpha4\alpha5$(IV) network is also present in cochlea and lens capsule. This explains why many AS patients can also present with sensorineural hearing loss and/or eye defects. Once the lens capsule becomes weak, the lens can herniate either anteriorly or posteriorly to cause lenticonus.

Immunohistologic analysis of the distribution of type IV collagen chains in renal and skin biopsies are crucial for the diagnosis of AS. In X-linked AS, no $\alpha3$, $\alpha4$, or $\alpha5$ deposition in the GBM is detectable in male patients because once $\alpha5$ is missing, the formation of the $\alpha3\alpha4\alpha5$(IV) protomer is disrupted. In contrast, the distribution of $\alpha3\alpha4\alpha5$(IV) trimers in the GBM is mosaic in a female carrier. In autosomal recessive AS, there is no $\alpha3$, $\alpha4$, or $\alpha5$ positive staining in the GBM. The $\alpha1$ and $\alpha2$ chains, which are normally confined to the mesangium and the subendothelial aspect of the GBM, are present throughout the entire width of the GBM in both forms of AS.

In X-linked AS, skin biopsy, which is much less invasive than kidney biopsy, can be highly informative because the $(\alpha5)_2\alpha6$ collagen IV network is normally present in the epidermal basement membrane. Thus, $\alpha5$ staining is absent from the epidermal basement membrane of male patients, whereas segmental staining of $\alpha5$ is seen in female carriers. In contrast, normal staining of the epidermal basement membrane is observed in patients with autosomal recessive or autosomal dominant AS.[31] If the suspicion of X-linked AS is high based on family history, a skin biopsy should be performed first. Otherwise, a renal biopsy should be performed.[32]

The characteristic electron microscopic findings of the GBM in AS are irregular thickening, thinning and basket weaving, which consists of irregular splitting of the lamina densa, giving rise to multiple thin interwoven lamellae separated by lucent space.[33] The molecular mechanisms underlying these ultrastructural changes are still obscure. It has been suggested that the thickened GBM may represent areas of matrix deposition.[34,35] Furthermore, it has been shown that the anomalous persistence of the fetal $\alpha1$(IV) and $\alpha2$(IV) isoforms in AS confers an increased susceptibility to endoproteolytic cleavage as compared to the more cysteine-rich $\alpha3\alpha4\alpha5$(IV) network in the mature GBM of normal kidneys; this may explain the progressive GBM splitting and increased deterioration.[36] Recently, it has been demonstrated that the proteolytic degradation of the GBM in $\alpha3$(IV) knockout Alport mice may be mediated, in part, by induction of matrix metalloproteinase-12 (MMP-12) in podocytes. It has also been suggested that monocyte chemoattractant protein -1 (MCP-1) activation of CC chemokine receptor 2 (CCR2) on podocytes may underlie the elevated expression of MMP-12.[37]

COLLAGEN IV AND ACQUIRED ANTI-GBM NEPHRITIS – GOODPASTURE'S DISEASE AND ALPORT POST-TRANSPLANTATION NEPHRITIS

There are two forms of anti-GBM nephritis: Goodpasture's disease and Alport post-transplantation nephritis (APTN). Goodpasture's disease is a disorder in which circulating autoantibodies attack the GBM and/or pulmonary alveolar BM, thereby causing rapidly progressive glomerulonephritis with crescent formation and/or pulmonary hemorrhage. Epitopes A and B (E_A and E_B), located in the $\alpha3$ chain NC1 domain, were identified as the Goodpasture autoantigen.[38-40] APTN occurs in 3% to 5% of Alport patients who receive renal transplants. The recipient can sometimes mount an alloimmune response against the normal type IV collagen existing in the normal donor kidney. However, the target antigen in APTN had been elusive.

Recent work from Billy Hudson's group has advanced our understanding of the pathogenesis of these two forms of antibody-mediated glomerulonephritis.[41] Besides E_A and E_B identified in $\alpha3$NC1, they discovered a new E_A region in the $\alpha5$ NC1 domain as a Goodpasture autoantigen. In addition, these investigators identified the E_A region in $\alpha5$ NC1 as the alloantigen in APTN. It is more intriguing that their new work showed a clear difference between neoepitopes in Goodpasture's disease and alloepitopes in APTN. The amino acid residues in neoepitopes are cryptic and sequestered in the quaternary structure of the hexamers. Disruption of the hexamer changes the conformation and exposes neoepitopes to elicit the production of Goodpasture autoantibodies. In sharp contrast, the amino acid residues in alloepitopes are exposed on the surface of the hexamer, and binding with APTN alloantibodies decreases upon dissociation of the hexamer. In Goodpasture's disease, they hypothesize that a variety of triggering events including post-translational modifications (oxidation, nitrosylation, and glycation), proteolytic cleavage and environmental factors such as cigarette smoking or exposure to organic solvents promote the conformational transition to a dissociated pathogenic conformer that elicits an autoimmune response.

HEPARAN SULFATE PROTEOGLYCANS

The charge selectivity of the GFB has been the subject of extensive investigations for decades. Heparan- and chondroitin-sulfate glycosaminoglycan (GAG) side chains of heparan sulfate proteoglycans (HSPGs) endow the GBM with its electronegative charge. Three distinct BM-HSPGs have been identified: perlecan, collagen XVIII, and agrin. Glomerular charge selectivity was attributed to the GBM's anionic sites enriched with HSPGs.[42-45] However, this concept has been challenged.

Perlecan and collagen XVIII are localized primarily to the MM and Bowman's capsule and are only prominent in the GBM during development.[46,47] Whereas perlecan mutant mice (*Hspg2*$^{\Delta3/\Delta3}$) lacking attachment sites for three heparan sulfate side chains exhibited no morphologic abnormalities in kidneys, they had a greater susceptibility to protein overload.[48,49] Gene ablation studies of collagen XVIII in mice showed that the mutants have mild MM expansion and slightly elevated serum creatinine levels compared with the controls. However, the study did not reveal any change of the GBM in the knockout mice.[50] Agrin is the predominant GBM-HSPG at maturity, and it is derived primarily from podocytes. Podocyte-specific knockout of agrin demonstrated that despite a marked reduction in the number of anionic sites in the GBM, the GFB was not compromised, even when challenged with albumin overload. These findings, thus, indicate that agrin is dispensable for the establishment or maintenance of the integrity of the GFB.[51] Moreover, in perlecan/agrin double mutants, the absence of perlecan-HS in combination with agrin did not cause proteinuria, and excretion of a negatively charged Ficoll tracer was unchanged as compared to that in wild type mice.[52] In line with these findings, reduction of anionic sites in the GBM by heparanase by using mice overexpressing heparanase did not lead to proteinuria.[53]

The charge selectivity of the GFB is highly controversial. In view of the recent *in vivo* studies, it appears that the GBM does not play a significant role in the charge-selective properties of the GFB. Recently, Axelsson et al[54] has demonstrated that the diffusion of conformationally identical anionic Ficoll across the rat GFB was significantly reduced compared with that of neutral Ficoll. This finding is in contrast with their previous finding of increased glomerular permeability of negatively charged Ficoll relative to neutral Ficoll.[55] Their explanation is that the charge modification of Ficoll in their previous study had significantly increased the molecular radius for all molecular weights, making it more flexible and hyperpermeable across the GFB compared with neutral Ficoll. Based on this finding and other experimental evidence indicating that the GFB does retard anionic proteins compared with neutral or cationic proteins,[56-58] they speculate that the charge selectivity of the GFB may reside in the endothelial glycocalyx.

NIDOGENS

Nidogens 1 and 2, also known as entactins 1 and 2, are BM glycoproteins expressed by distinct genes located on different chromosomes.[59-61] They bind tightly to the laminin $\gamma1$ chain short arm and also bind to type IV collagen. Therefore, previously it was thought that nidogens are the major molecular link between the laminin and collagen IV networks and should, therefore, be crucial for BM formation. However, this notion is not supported by the gene deletion studies. The loss of either isoform had no effect on BM formation and organ development.[62,63] However, genetic ablation of both nidogens 1 and 2 in mice resulted in perinatal lethality, which was associated with impaired lung and cardiac development. But despite the ubiquitous presence of nidogens in basement membranes, most of the nidogen double-null mice had developed functioning kidneys with well-defined GBM, suggesting tissue-specific roles of nidogen in the formation and maintenance of certain basement membranes.[64]

CONCLUSION

Various studies have been performed to delineate whether the GBM or the slit diaphragm (SD), a cell-cell junction spanning the spaces between the interdigitating foot processes, is the primary filtration barrier of kidney. In recent years, the composition and functions of the SD have been extensively investigated. However, the GBM should not be overlooked. To maintain the integrity of the GFB, it appears that the laminin and type IV collagen networks of the GBM are critical, while HSPG and nidogen are dispensable.

For several decades, the GBM has been envisioned as being a charge-selective barrier for glomerular filtration. However, the recent genetic studies including agrin deletion and compound agrin/perlecan deletion in the GBM have challenged the traditional concept. It seems that the GBM does not contribute much to the charge selectivity of the GFB. It has been postulated that the endothelial cell layer may exert more pronounced effect on the charge selectivity of the GFB. Further experimentation will be required to elucidate the exact mechanisms of the charge-selectivity of the GFB.

In conclusion, all strata of the glomerular capillary wall including GBM, podocytes and endothelial cells operate in a synchronic and integrated manner to maintain the permselectivity of the GFB.

REFERENCES

1. Abrahamson DR. Origin of the glomerular basement membrane visualized after *in vivo* labeling of laminin in newborn rat kidneys. J Cell Biol 1985;100:1988–2000.
2. Sasaki T, Fassler R, Hohenester E. Laminin: the crux of basement membrane assembly. J Cell Biol 2004;164:959–63.
3. Aumailley M, Bruckner-Tuderman L, Carter WG, et al. A simplified laminin nomenclature. Matrix Biol 2005;24:326–32.
4. St John PL, Abrahamson DR. Glomerular endothelial cells and podocytes jointly synthesize laminin-1 and -11 chains. Kidney Int 2001;60:1037–46.
5. Miner JH, Patton BL, Lentz SI, et al. The laminin α chains: expression, developmental transitions, and chromosomal locations of α-5, identification of heterotrimeric laminins 8-11, and cloning of a novel α3 isoform. J Cell Biol 1997;137:685–701.

6. Miner JH, Sanes JR. Collagen IV α3, α4, and α5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. J Cell Biol 1994;127:879–91.
7. Miner JH. Building the glomerulus: a matricentric view. J Am Soc Nephrol 2005;16:857–61.
8. McKee KK, Harrison D, Capizzi S, et al. Role of laminin terminal globular domains in basement membrane assembly. J Biol Chem 2007;282:21437–47.
9. Colognato H, Winkelmann DA, Yurchenco PD. Laminin polymerization induces a receptor-cytoskeleton network. J Cell Biol 1999;145:619–31.
10. Zenker M, Aigner T, Wendler O, et al. Human laminin β2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. Hum Mol Genet 2004;13:2625–32.
11. Zenker M, Tralau T, Lennert T, et al. Congenital nephrosis, mesangial sclerosis, and distinct eye abnormalities with microcoria: an autosomal recessive syndrome. Am J Med Genet A 2004; 130A:138–45.
12. Zenker M, Pierson M, Jonveaux P, et al. Demonstration of two novel LAMB2 mutations in the original Pierson syndrome family reported 42 years ago. Am J Med Genet A 2005;138: 73–4.
13. Matejas V, Hinkes B, Alkandari F, et al. Mutations in the human laminin β2 (LAMB2) gene and the associated phenotypic spectrum. Hum Mutat 2010;31:992–1002.
14. Noakes PG, Miner JH, Gautam M, et al. The renal glomerulus of mice lacking s-laminin/laminin β2: nephrosis despite molecular compensation by laminin β1. Nat Genet 1995;10:400–6.
15. Libby RT, Lavallee CR, Balkema GW, et al. Disruption of laminin β2 chain production causes alterations in morphology and function in the CNS. J Neurosci 1999;19:9399–411.
16. Knight D, Tolley LK, Kim DK, et al. Functional analysis of neurotransmission at β2-laminin deficient terminals. J Physiol 2003; 546:789–800.
17. Nishimune H, Sanes JR, Carlson SS. A synaptic laminin-calcium channel interaction organizes active zones in motor nerve terminals. Nature 2004;432:580–7.
18. Noakes PG, Gautam M, Mudd J, et al. Aberrant differentiation of neuromuscular junctions in mice lacking s-laminin/laminin β2. Nature 1995;374:258–62.
19. Patton BL, Chiu AY, Sanes JR. Synaptic laminin prevents glial entry into the synaptic cleft. Nature 1998;393:698–701.
20. Hasselbacher K, Wiggins RC, Matejas V, et al. Recessive missense mutations in LAMB2 expand the clinical spectrum of LAMB2-associated disorders. Kidney Int 2006;70:1008–12.
21. Matejas V, Hinkes B, Alkandari F, et al. Mutations in the human laminin β2 (LAMB2) gene and the associated phenotypic spectrum. Hum Mutat 2010;31:992–1002.
22. Chen YM, Kikkawa Y, Miner JH. A missense LAMB2 mutation causes congenital nephrotic syndrome by impairing laminin secretion. J Am Soc Nephrol 2011;22:849–58.
23. Goldberg S, Adair-Kirk TL, Senior RM, et al. Maintenance of glomerular filtration barrier integrity requires laminin α5. J Am Soc Nephrol 2010;21:579–86.
24. Khoshnoodi J, Cartailler JP, Alvares K, et al. Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. J Biol Chem 2006;281:38117–21.
25. Hudson BG, Tryggvason K, Sundaramoorthy M, et al. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med 2003;348:2543–56.
26. Abrahamson DR, Hudson BG, Stroganova L, et al. Cellular origins of type IV collagen networks in developing glomeruli. J Am Soc Nephrol 2009;20:1471–9.
27. Heidet L, Cai Y, Guicharnaud L, et al. Glomerular expression of type IV collagen chains in normal and X-linked Alport syndrome kidneys. Am J Pathol 2000;156:1901–10.
28. Kashtan CE. Familial hematuria due to type IV collagen mutations: Alport syndrome and thin basement membrane nephropathy. Curr Opin Pediatr 2004;16:177–81.
29. Haas M. Alport syndrome and thin glomerular basement membrane nephropathy: a practical approach to diagnosis. Arch Pathol Lab Med 2009;133:224–32.
30. Piccini M, Casari G, Zhou J, et al. Evidence for genetic heterogeneity in benign familial hematuria. Am J Nephrol 1999;19:464–7.
31. Gubler MC. Inherited diseases of the glomerular basement membrane. Nat Clin Pract Nephrol 2008;4:24–37.
32. Kashtan CE. Familial hematuria. Pediatr Nephrol 2009;24:1951–8.
33. Spear GS, Slusser RJ. Alport's syndrome. Emphasizing electron microscopic studies of the glomerulus. Am J Pathol 1972;69:213–24.
34. Cosgrove D, Rodgers K, Meehan D, et al. Integrin $\alpha1\beta1$ and transforming growth factor-β1 play distinct roles in alport glomerular pathogenesis and serve as dual targets for metabolic therapy. Am J Pathol 2000;157:1649–59.
35. Abrahamson DR, Prettyman AC, Robert B, et al. Laminin-1 reexpression in Alport mouse glomerular basement membranes. Kidney Int 2003;63:826–34.
36. Kalluri R, Shield CF, Todd P, et al. 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;99:2470–8.
37. Rao VH, Meehan DT, Delimont D, et al. Role for macrophage metalloelastase in glomerular basement membrane damage associated with alport syndrome. Am J Pathol 2006;169:32–46.
38. Turner N, Mason PJ, Brown R, et al. Molecular cloning of the human Goodpasture antigen demonstrates it to be the α3 chain of type IV collagen. J Clin Invest 1992;89:592–601.
39. Saus J, Wieslander J, Langeveld JP, et al. Identification of the Goodpasture antigen as the α 3(IV) chain of collagen IV. J Biol Chem 1988;263:13374–80.
40. Butkowski RJ, Langeveld JP, Wieslander J, et al. Localization of the Goodpasture epitope to a novel chain of basement membrane collagen. J Biol Chem 1987;262:7874–7.
41. Pedchenko V, Bondar O, Fogo AB, et al. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. N Engl J Med 2010;363:343–54.
42. Rennke HG, Cotran RS, Venkatachalam MA. Role of molecular charge in glomerular permeability. Tracer studies with cationized ferritins. J Cell Biol 1975;67:638–46.
43. Brenner BM, Hostetter TH, Humes HD. Molecular basis of proteinuria of glomerular origin. N Engl J Med 1978;298:826–33.
44. Kanwar YS, Linker A, Farquhar MG. Increased permeability of the glomerular basement membrane to ferritin after removal of glycosaminoglycans (heparan sulfate) by enzyme digestion. J Cell Biol 1980;86:688–93.
45. Kanwar YS, Farquhar MG. Presence of heparan sulfate in the glomerular basement membrane. Proc Natl Acad Sci U S A 1979;76:1303–7.
46. Groffen AJ, Veerkamp JH, Monnens LA, et al. Recent insights into the structure and functions of heparan sulfate proteoglycans in the human glomerular basement membrane. Nephrol Dial Transplant 1999;14:2119–29.
47. Saarela J, Rehn M, Oikarinen A, et al. The short and long forms of type XVIII collagen show clear tissue specificities in their expression and location in basement membrane zones in humans. Am J Pathol 1998;153:611–26.
48. Rossi M, Morita H, Sormunen R, et al. Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. EMBO J 2003;22:236–45.

49. Morita H, Yoshimura A, Inui K, et al. Heparan sulfate of perlecan is involved in glomerular filtration. J Am Soc Nephrol 2005;16: 1703–10.
50. Utriainen A, Sormunen R, Kettunen M, et al. Structurally altered basement membranes and hydrocephalus in a type XVIII collagen deficient mouse line. Hum Mol Genet 2004;13:2089–99.
51. Harvey SJ, Jarad G, Cunningham J, et al. Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. Am J Pathol 2007;171:139–52.
52. Goldberg S, Harvey SJ, Cunningham J, et al. Glomerular filtration is normal in the absence of both agrin and perlecan-heparan sulfate from the glomerular basement membrane. Nephrol Dial Transplant 2009;24:2044–51.
53. van den Hoven MJ, Wijnhoven TJ, Li JP, et al. Reduction of anionic sites in the glomerular basement membrane by heparanase does not lead to proteinuria. Kidney Int 2008;73:278–87.
54. Axelsson J, Sverrisson K, Rippe A, et al. Reduced diffusion of charge-modified, conformationally intact anionic Ficoll relative to neutral Ficoll across the rat glomerular filtration barrier in vivo. Am J Physiol Renal Physiol 2011;301:F708–12.
55. Asgeirsson D, Venturoli D, Rippe B, et al. Increased glomerular permeability to negatively charged Ficoll relative to neutral Ficoll in rats. Am J Physiol Renal Physiol 2006;291:F1083–9.
56. Comper WD, Glasgow EF. Charge selectivity in kidney ultrafiltration. Kidney Int 1995;47:1242–51.
57. Lund U, Rippe A, Venturoli D, et al. Glomerular filtration rate dependence of sieving of albumin and some neutral proteins in rat kidneys. Am J Physiol Renal Physiol 2003;284:F1226–34.
58. Venturoli D, Rippe B. Ficoll and dextran vs globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability. Am J Physiol Renal Physiol 2005;288:F605–13.
59. Timpl R, Dziadek M, Fujiwara S, et al. Nidogen: a new, self-aggregating basement membrane protein. Eur J Biochem 1983; 137:455–65.
60. Kohfeldt E, Sasaki T, Gohring W, et al. Nidogen-2: a new basement membrane protein with diverse binding properties. J Mol Biol 1998;282:99–109.
61. Zimmermann K, Hoischen S, Hafner M, et al. Genomic sequences and structural organization of the human nidogen gene (NID). Genomics 1995;27:245–50.
62. Murshed M, Smyth N, Miosge N, et al. The absence of nidogen 1 does not affect murine basement membrane formation. Mol Cell Biol 2000;20:7007–12.
63. Schymeinsky J, Nedbal S, Miosge N, et al. Gene structure and functional analysis of the mouse nidogen-2 gene: nidogen-2 is not essential for basement membrane formation in mice. Mol Cell Biol 2002;22:6820–30.
64. Bader BL, Smyth N, Nedbal S, et al. Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. Mol Cell Biol 2005;25:6846–56.