# Proteinuria with and without Renal Glomerular Podocyte Effacement

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**Renal biopsies of patients with proteinuria and kidney disease most often are associated with podocyte foot process effacement. For several decades, nephrologists have wondered whether proteinuria is a result of podocyte foot process effacement or the cause of it. In the past few years, the author's laboratory has addressed this issue using different mouse models of proteinuria. Although in most cases, podocyte effacement is associated with proteinuria and glomerular disease, in three different mouse models, it was demonstrated that proteinuria can be observed without podocyte foot process effacement. The first model is generated by injection of antibodies to vascular endothelial growth factor or soluble vascular endothelial growth factor receptor 1. The second model is a mouse with deletion of type IV collagen α3 chain in the glomerular basement membrane. The third model was generated by genetic deletion of a slit diaphragm protein known as nephrin. Collectively, these experiments and the supporting evidence from several human studies demonstrate that severe defects in either the glomerular basement membrane or the glomerular endothelium can lead to proteinuria without foot process effacement.**

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In the process of blood filtration, the two kidneys in the human body clear approximately 125 ml of filtrate that enters the renal tubular system *via* the glomeruli every minute, or approximately 180 L of plasma filtrate every day. In a normal physiologic setting, it is thought at any given time, the filtrate volume represents approximately 20% of the total plasma that enters the glomeruli, and the other 80% of the plasma bypasses the renal tubular system and enters the efferent arterioles directly. Of 125 ml of filtrate that enters the renal tubular system per minute, 124 ml is reclaimed by tubular resorption, tubular secretion, and concentration. The urine that enters the ureters is very different in its molecular composition and is only a very small fraction of the total plasma being filtered, which in a given day can accumulate to approximately 1.5 L in the bladder. During a 24-h period, approximately 1.5 g of salt also is filtered by the renal system.

Renal glomerular filtration apparatus is composed of three different components: The fenestrated endothelium, the glomerular basement membrane (GBM), and the epithelial cells with characteristic foot processes, known as podocytes (1–9). Filtration through this barrier depends on the charge and the size of the molecules present in the blood. In addition, it depends on the net filtration pressure in the glomerular tuft. The entire blood volume of a human passes through the kidneys approximately every 5 min. In a normal physiologic setting, the arterial capillaries of the glomerular tuft are under a hydrostatic pressure of approximately 45 mmHg (this pressure is higher than that found in other capillaries) and facilitated by the juxtaglomerular apparatus autoregulation, which constricts or dilates the afferent arterioles in response to changes in BP, and also *via* the regulation of the renin-angiotensin system. Conceptually, the glomerular filtrate is formed in response to the hydrostatic pressure of blood, which is partially opposed by the osmotic pressure of the plasma colloids (20 mmHg), and also by the hydrostatic pressure of the fluids in the Bowman's capsule (10 mmHg). The resultant net filtration pressure in the glomerular capillaries is approximately 15 mmHg. Vasoconstriction of the efferent arterioles can lead to hypertension and glomerular hyperfiltration, potentially leading to hyperpermeability of albumin (10–12). Therefore, angiotensin-converting enzyme inhibitors are used widely in the nephrology clinic to decrease glomerular hyperfiltration by decreasing BP and hence decreasing albuminuria (13).

The fenestrated endothelium is considered to be a barrier for large cells and aggregated cells in the blood, but most proteins and solutes pass through the endothelial layer. The GBM is approximately 300 nm thick and contains proteins such as fibronectin, negatively charged heparan sulfate proteoglycans, type IV collagens, laminins, *etc.* (14–16). The GBM acts as a physical filter and also as a charger barrier. Molecules >10 nm in diameter do not cross the GBM, and the negatively charged proteins of >70 kD can minimally cross GBM (17).

The interdigitating foot processes of the podocytes from two podocyte cell bodies associate *via* a modified adherens junction complex, known as the slit diaphragm, of approximately 6 nm thick (18–20). Several proteins have been discovered recently

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within the slit diaphragm, and they associate with each other and are connected directly or indirectly to the actin filaments of the podocytes and regulate its function (19,21). An understanding of the precise function of podocytes still is evolving, and much more work needs to be done.

Debates about which one of the three components of the glomerular filtration apparatus is the defining barrier that keeps albumin from escaping from the blood have been going on for many years (1,9). It now is generally believed that the charge barrier of the GBM may not be the most prominent filter, but the composite effect of the GBM charge and also slit diaphragm integrity are essential for the successful retention of albumin and other proteins that are >70 kD. Under normal physiologic conditions, how the trapped albumin in the GBM and the slit diaphragm returns back into the circulation still is not understood, but many theories exist. Tubular and glomerular reabsorption, cellular endocytosis, and an active glomerular reflow into the blood in the reverse direction from the slit diaphragm against flow and pressure are all a possibility. Collectively, evidence gathered from our laboratory in the past few years suggest that damage to any of the three components of glomerular filtration apparatus results in proteinuria without effacement of podocyte foot processes.

Vascular endothelial growth factor (VEGF) is a key endothelial survival factor and induces vascular permeability (22–24). We tested the hypothesis of whether circulating physiologic levels of VEGF can provide survival cues to the glomerular endothelial cells and help maintain the fenestrations. The motivation for these experiments also stems from observations in oncology clinics that in a significant percentage of patients, anti-VEGF antibody therapy leads to proteinuria and hypertension (25,26). In addition, between 1998 and 2002, several reports indicated that women with preeclampsia (who among other things exhibit proteinuria and hypertension) present with elevated levels of soluble VEGF receptor 1 (sFLT-1), detected as an increase in the amniotic fluid and cytotrophoblasts (27–29). To test this clinical observation experimentally, we neutralized circulating VEGF in mice using equimolar amounts of mouse anti-VEGF antibody or sFLT-1 (30). In these experiments, we observed that proteinuria can be induced by neutralizing circulating VEGF without altering the levels of endogenous kidney tissue associated VEGF (30). Interesting, we demonstrate that proteinuria can be induced in these mice with anti-VEGF antibody and sFLT-1 without podocyte foot process effacement (30) (Figure 1). Predominant lesions observed in these mice are glomerular endothelial damage, endotheliosis with large vacuoles, and detachment from the GBM, resembling the histopathology that is observed in women with preeclampsia (30).

Predominant components of GBM are type IV collagen and laminin (15). The predominant type IV collagen constituents of GBM proper are $\alpha$3, $\alpha$4, and $\alpha$5 chain (31). Therefore, deletion of $\alpha$3 chain of type IV collagen ($\alpha$3KO) in mice leads to severe GBM defects as a result of elimination of all three chains of type IV collagen due to obligatory assembly that is required among the type IV collagen chains (14,32). Electron microscopy (EM) pictures show significant defects in the GBM, early in the life of the $\alpha$3KO mice on the 129/sv background (1) (Figure 2). By approximately 5 wk, these mice develop proteinuria, and careful EM examination of glomerular architecture reveals intact endothelial layer, significant GBM defects (splitting, thinning, basketweave pattern, and thickening), and intact podocyte foot processes (Figure 2). Continued proteinuria with time results in podocyte effacement in these mice (Figure 2). The $\alpha$3KO mice are a model for autosomal recessive Alport syndrome, and our results suggest that early hematuria and albuminuria that are seen in these patients could occur without podocyte foot process effacement. Our results with the $\alpha$3KO mice demonstrate for the first time that significant GBM structural and functional defects can lead to massive proteinuria in mice without podocyte foot process effacement.

Nephrin is a component of the podocyte slit diaphragm and also of other structures and cellular constituents of the body, including the nervous system (33,34). Mutations in nephrin have been identified in patients with nephrotic syndrome of the Finnish type (33). We generated mice that are deficient in nephrin, and these mice die at approximately 2 d after birth and are associated with massive proteinuria. We are not sure whether the phrase "nephrotic-range proteinuria" should be used in the context of mice; therefore, we use the term "massive proteinuria" here. This massive proteinuria in mice occurs without obvious podocyte foot process effacement (Figure 3). It is not clear yet whether the early death that is seen in these mice is due to the kidney phenotype or due to some other, unknown defects (1). Other investigators also have demonstrated that targeting nephrin can lead to slit diaphragm defects (35) and proteinuria without significant podocyte foot process effacement (36–38). Collectively, studies with nephrin-deficient mice demonstrate that massive proteinuria can be observed without any defects in the GBM, glomerular endothelium, or podocyte foot processes (Figure 3).

Our findings demonstrate that defects that are induced in any of the three components of the glomerular filtration apparatus can lead to initial proteinuria without podocyte foot process effacement. Sustained proteinuria, eventually in all three settings, is associated with podocyte foot process effacement, so what causes proteinuria without podocyte effacement? Our contention is that all three components of the glomerular filtration apparatus are in constant molecular and biochemical communication with each other, *via* GBM–cell interactions and possibly also *via* growth factor and other soluble ligand-receptor influences (Figure 4). Therefore, although gross morphologic changes in the podocyte foot process may not be observed during the early phases of the abnormal protein leak in the urine, subtle molecular alterations in the slit diaphragm composition, assembly, and signaling are possible at this stage without overt morphologic changes (Figure 4). Subsequently, sustained insult/injury potentially leads to an eventual overt morphologic defect, observed as podocyte foot process effacement. Foot process effacement is associated with an enhanced generalized, nonspecific adhesion that engages neighboring foot processes and a loss of well-organized adherens junctions (slit diaphragms). Such defects potentially lead to loss of specific signaling pathways and compromised functional adhesion to the GBM. Therefore, what causes proteinuria still is an

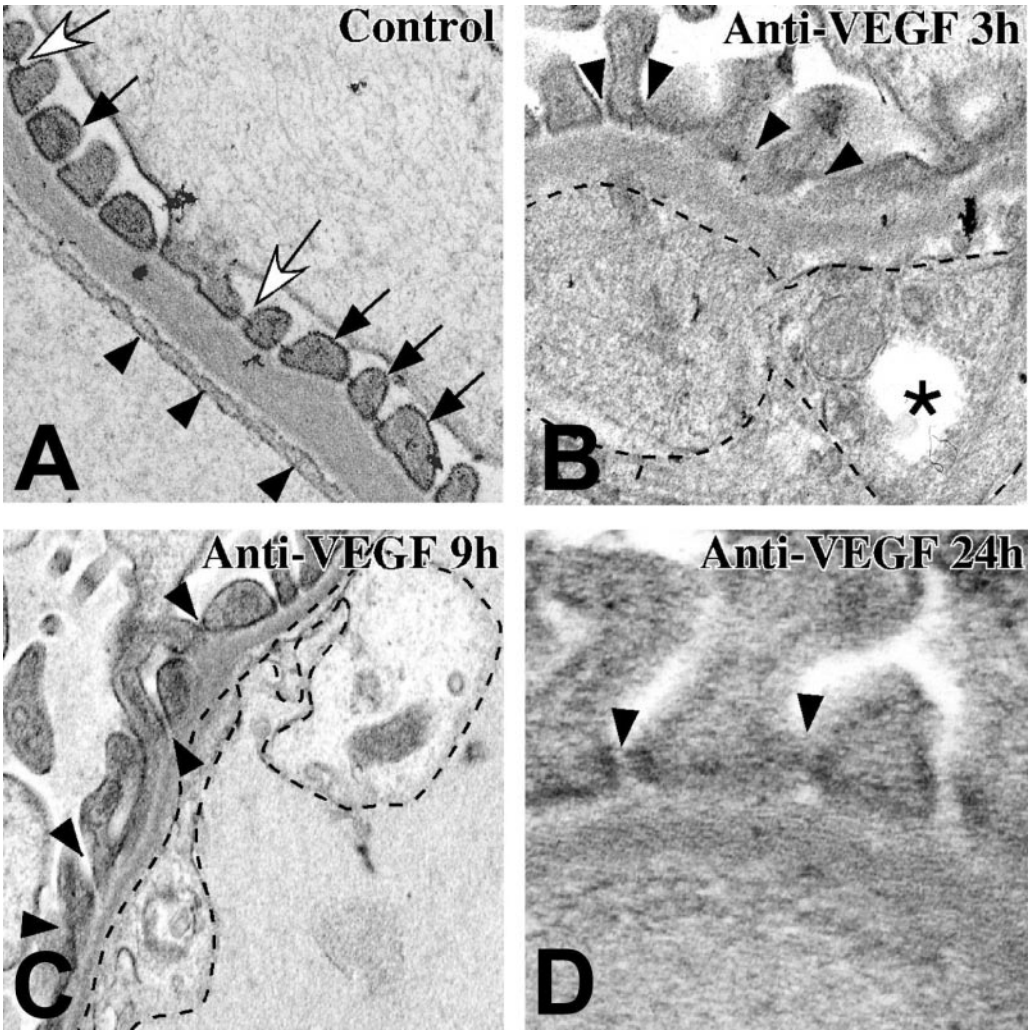


*Figure 1.* Neutralizing circulating vascular endothelial growth factor (VEGF) leads to proteinuria without podocyte foot process effacement. (A) Control mice. (B) Endotheliosis observed in mice upon anti-VEGF antibody administration. (C) Same as B but at 9 h. (D) Same as B and C but at 24 h, demonstrating intact foot processes. These data were originally reported by Sugimoto *et al.* (30). Magnifications: ×30,000 in A; ×21,400 in B and C; ×67,000 in D.

open-ended question. Nevertheless, it is clear now that podocyte foot process effacement is not required for initiation of proteinuria. Defects that are induced in the glomerular endothelial cells, GBM, or the slit diaphragm can lead to proteinuria without podocyte foot process effacement. In this regard, several other studies in mice and rats have demonstrated that proteinuria can be observed without podocyte foot process effacement, supporting the studies documented here (36,39,40). Most interesting, male MWF rats develop spontaneous proteinuria with age but without podocyte foot process effacement (41).

In the 1950s, Farquhar *et al.* (42,43) first described patients with nephrosis, glomerulonephritis, and lupus erythematosus with extensive podocyte foot process effacement. Since then, several other human studies advance the notion that proteinuria can occur without obvious podocyte foot process effacement (44–47). Van den Berg *et al.* (44) documented in elegant studies that podocyte foot process is not correlated with the level of proteinuria in several human glomerulopathies. Variants of minimal-change nephritic syndrome with proteinuria are not associated with podocyte foot process effacement (44). Branten *et al.* (45) report that a familial form of nephrotic syndrome occurs in the absence of podocyte foot process effacements. Additionally, several other anecdotal reports with human biopsies that support the notion that proteinuria can occur without podocyte foot process effacement exist. It is interesting that the most convincing of such reports have been around for a few decades now. These include EM studies of the kidney glomeruli of women with preeclampsia, a syndrome that is associated with proteinuria and hypertension and is seen in approximately 5% of pregnant women (48,49). Proteinuria, hypertension, and glomerular endotheliosis in these women are not associated with podocyte foot process effacement (Figure 5).

Proteinuria still represents a key biomarker for kidney dysfunction. What causes excessive protein leak and albuminuria

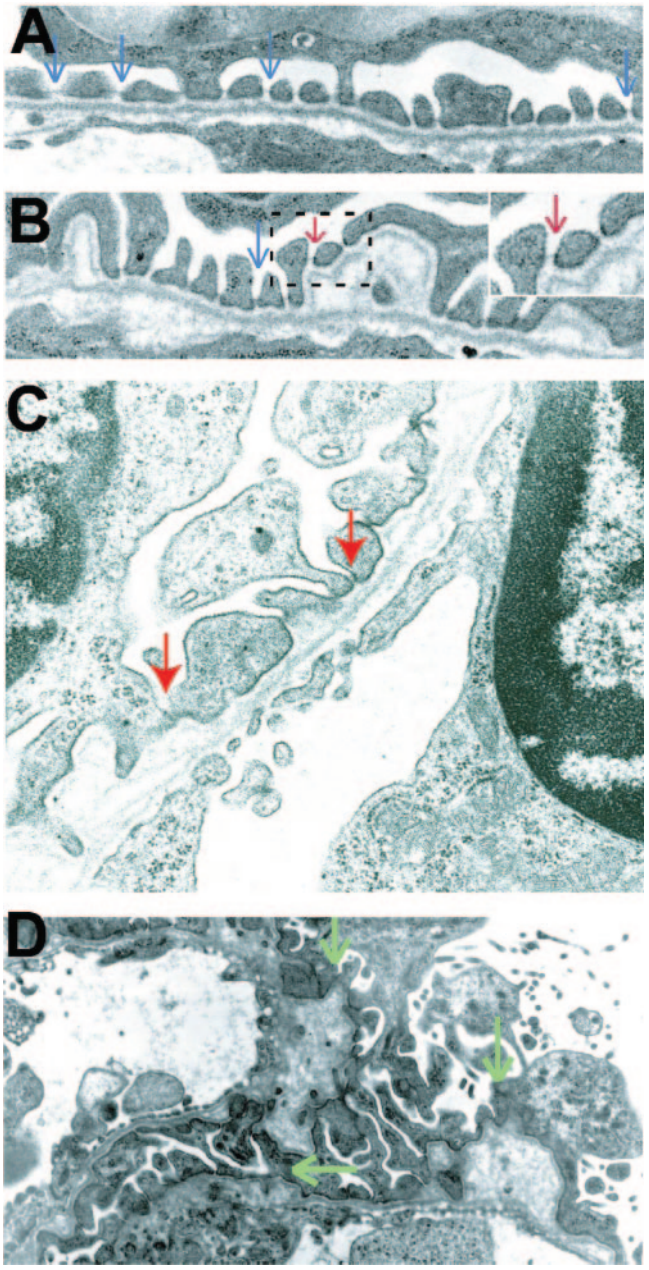


*Figure 2.* Transmission electron microscope analysis of the α3KO kidneys with and without proteinuria. (A) Control wild-type kidney at 4 wk of age. Illustrates normal glomerular basement membrane (GBM) architecture. (B) Nonproteinuric 4-wk-old α3KO mice with significant GBM defects, normal glomerular endothelial cells, and normal podocyte foot processes. (C) Proteinuric 5-wk-old α3KO mice with GBM defects, normal glomerular endothelial cells, and normal podocyte foot processes. (D) Proteinuric 8-wk-old α3KO mice with GBM defects, mild to moderate glomerular endothelial damage, and significant podocyte foot process effacement. These data were originally reported by Hamano *et al.* (1), except for panel C. Magnifications: ×35,000 in A and B; ×30,000 in C; ×12,250 in D.

is not yet known. Therefore, experiments with a mechanistic focus on what causes proteinuria still might represent the best approach to identifying biomarkers for most renal diseases.

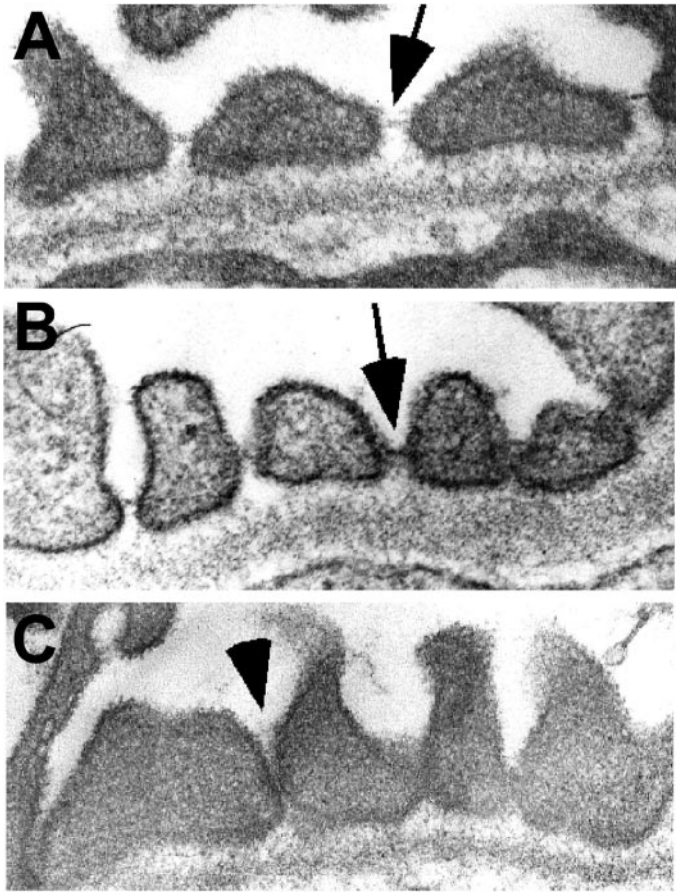


*Figure 3.* Transmission electron microscope analysis of glomeruli of nephrin-deficient mice. (A) Wild-type mice at day 2 after birth. (B) Heterozygote mice at day 2 after birth. (C) Nephrin −/− mice at day 2 after birth with massive proteinuria but without podocyte foot process effacement. These data were originally presented in by Hamano *et al.* (1). Magnifications: ×180,000 in A and B; ×115,000 in C.

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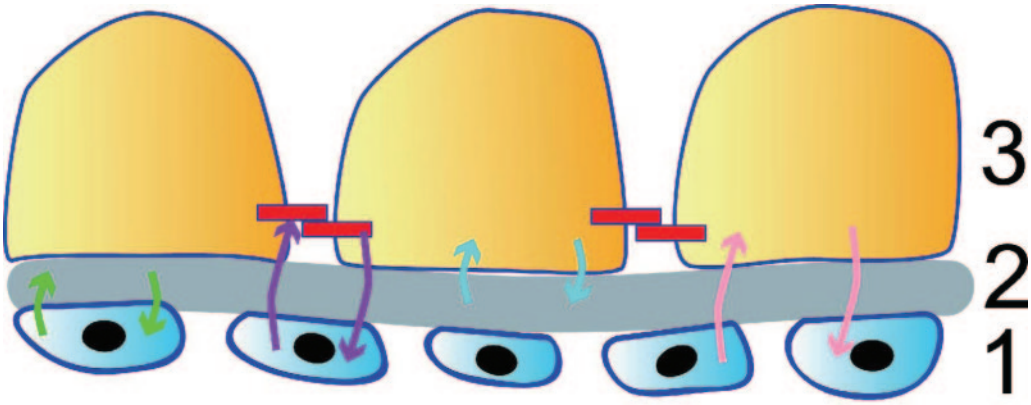


*Figure 4.* Renal glomerular filtration apparatus. The three components of the filtration apparatus are presented here. 1, Fenestrated endothelium; 2, GBM; 3, podocyte foot processes and the slit diaphragm. All of these components likely are in constant two-way molecular and biochemical communications with each other (arrows).

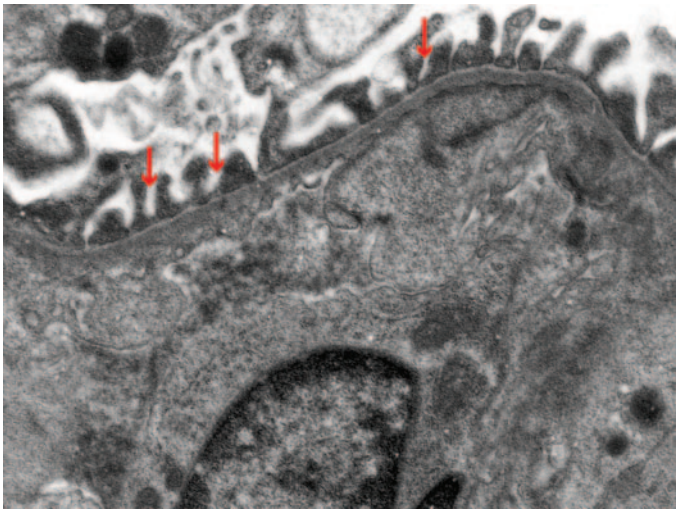

*Figure 5.* Renal biopsy of a 30-yr-old woman with twin pregnancy, who presented at 15 wk with new-onset hypertension and nephrotic-range proteinuria. Electron micrograph of a representative capillary loop showing lumenal occlusion by marked endothelial swelling (endotheliosis). Note that the podocyte foot processes are well preserved. Magnification, ×7500. Image courtesy of Dr. Isaac E. Stillman, Department of Pathology, BIDMC.

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## References

1. Hamano Y, Grunkemeyer JA, Sudhakar A, Zeisberg M, Cosgrove D, Morello R, Lee B, Sugimoto H, Kalluri R: Determinants of vascular permeability in the kidney glomerulus. *J Biol Chem* 30: 30, 2002
2. Salant DJ: The structural biology of glomerular epithelial cells in proteinuric diseases. *Curr Opin Nephrol Hypertens* 3: 569–574, 1994
3. Kerjaschki D: Dysfunctions of cell biological mechanisms of visceral epithelial cell (podocytes) in glomerular diseases. *Kidney Int* 45: 300–313, 1994
4. Karnovsky MJ, Ainsworth SK: The structural basis of glomerular filtration. *Adv Nephrol Necker Hosp* 2: 35–60, 1972
5. Kaysen GA, Myers BD, Couser WG, Rabkin R, Felts JM: Mechanisms and consequences of proteinuria. *Lab Invest* 54: 479–498, 1986
6. Kerjaschki D: Caught flat-footed: Podocyte damage and the molecular bases of focal glomerulosclerosis. *J Clin Invest* 108: 1583–1587, 2001
7. Kojima K, Davidovits A, Poczewski H, Langer B, Uchida S, Nagy-Bojarski K, Hovorka A, Sedivy R, Kerjaschki D: Podocyte flattening and disorder of glomerular basement membrane are associated with splitting of dystroglycan-matrix interaction. *J Am Soc Nephrol* 15: 2079–2089, 2004
8. Couser WG: Mechanisms of glomerular injury: An overview. *Semin Nephrol* 11: 254–258, 1991
9. Farquhar MG: Editorial: The primary glomerular filtration barrier—basement membrane or epithelial slits? *Kidney Int* 8: 197–211, 1975
10. Brenner BM: Retarding the progression of renal disease. *Kidney Int* 64: 370–378, 2003

11. Tripathi S, Tripathi K: Role of kidney in hypertension. *J Indian Med Assoc* 101: 260–262, 2003
12. Schieppati A, Remuzzi G: The June 2003 Barry M. Brenner Comgan lecture. The future of renoprotection: Frustration and promises. *Kidney Int* 64: 1947–1955, 2003
13. Brenner BM: Regarding: Management of glomerular proteinuria: A commentary. *J Am Soc Nephrol* 15: 1354–1355; discussion 1356–1357, 2004
14. Kalluri R, Cosgrove D: Assembly of type IV collagen. Insights from alpha3(IV) collagen-deficient mice. *J Biol Chem* 275: 12719–12724, 2000
15. Kalluri R: Basement membranes: Structure, assembly and role in tumour angiogenesis. *Nat Rev Cancer* 3: 422–433, 2003
16. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG: Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N Engl J Med* 348: 2543–2556, 2003
17. Kanwar YS, Farquhar MG: Anionic sites in the glomerular basement membrane. In vivo and in vitro localization to the laminae rarae by cationic probes. *J Cell Biol* 81: 137–153, 1979
18. Mundel P, Schwarz K, Reiser J: Podocyte biology: A footstep further. *Adv Nephrol Necker Hosp* 31: 235–241, 2001
19. Reiser J, Kriz W, Kretzler M, Mundel P: The glomerular slit diaphragm is a modified adherens junction. *J Am Soc Nephrol* 11: 1–8, 2000
20. Mundel P, Shankland SJ: Podocyte biology and response to injury. *J Am Soc Nephrol* 13: 3005–3015, 2002
21. Moller CC, Pollak MR, Reiser J: The genetic basis of human glomerular disease. *Adv Chronic Kidney Dis* 13: 166–173, 2006
22. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF: Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* 219: 983–985, 1983
23. Dvorak HF: Discovery of vascular permeability factor (VPF). *Exp Cell Res* 312: 522–526, 2006
24. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N: Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246: 1306–1309, 1989
25. Gordon MS, Margolin K, Talpaz M, Sledge GW Jr, Holmgren E, Benjamin R, Stalter S, Shak S, Adelman D: Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. *J Clin Oncol* 19: 843–850, 2001
26. Margolin K, Gordon MS, Holmgren E, Gaudreault J, Novotny W, Fyfe G, Adelman D, Stalter S, Breed J: Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: Pharmacologic and long-term safety data. *J Clin Oncol* 19: 851–856, 2001
27. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS: A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod* 59: 1540–1548, 1998
28. Vuorela P, Helske S, Hornig C, Alitalo K, Weich H, Halmesmaki E: Amniotic fluid–soluble vascular endothelial growth factor receptor-1 in preeclampsia. *Obstet Gynecol* 95: 353–357, 2000
29. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, Alitalo K, Damsky C, Fisher SJ: Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol* 160: 1405–1423, 2002
30. Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, Kalluri R: Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem* 278: 12605–12608, 2003
31. 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* 99: 2470–2478, 1997
32. 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* 10: 2981–2992, 1996
33. Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K: Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1: 575–582, 1998
34. Tryggvason K, Wartiovaara J: Molecular basis of glomerular permselectivity. *Curr Opin Nephrol Hypertens* 10: 543–549, 2001
35. Rantanen M, Palmen T, Patari A, Ahola H, Lehtonen S, Astrom E, Floss T, Vauti F, Wurst W, Ruiz P, Kerjaschki D, Holthofer H: Nephrin TRAP mice lack slit diaphragms and show fibrotic glomeruli and cystic tubular lesions. *J Am Soc Nephrol* 13: 1586–1594, 2002
36. Topham PS, Kawachi H, Haydar SA, Chugh S, Addona TA, Charron KB, Holzman LB, Shia M, Shimizu F, Salant DJ: Nephritogenic mAb 5-1-6 is directed at the extracellular domain of rat nephrin. *J Clin Invest* 104: 1559–1566, 1999
37. Orikasa M, Matsui K, Oite T, Shimizu F: Massive proteinuria induced in rats by a single intravenous injection of a monoclonal antibody. *J Immunol* 141: 807–814, 1988
38. Fujigaki Y, Morioka T, Matsui K, Kawachi H, Orikasa M, Oite T, Shimizu F, Batsford SR, Vogt A: Structural continuity of filtration slit (slit diaphragm) to plasma membrane of podocyte. *Kidney Int* 50: 54–62, 1996
39. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 111: 649–658, 2003
40. Kreisberg JI, Wayne DB, Karnovsky MJ: Rapid and focal loss of negative charge associated with mononuclear cell infiltration early in nephrotoxic serum nephritis. *Kidney Int* 16: 290–300, 1979
41. Macconi D, Ghilardi M, Bonassi ME, Mohamed EI, Abbate M, Colombi F, Remuzzi G, Remuzzi A: Effect of angiotensin-converting enzyme inhibition on glomerular basement membrane permeability and distribution of zonula occludens-1 in MWF rats. *J Am Soc Nephrol* 11: 477–489, 2000
42. Farquhar MG, Vernier RL, Good RA: An electron microscope study of the glomerulus in nephrosis, glomerulonephritis, and lupus erythematosus. *J Exp Med* 106: 649–660, 1957

43. Farquhar MG, Vernier RL, Good RA: The application of electron microscopy in pathology: Study of renal biopsy tissues. *Schweiz Med Wochenschr* 87: 501–510, 1957
44. van den Berg JG, van den Bergh Weerman MA, Assmann KJ, Weening JJ, Florquin S: Podocyte foot process effacement is not correlated with the level of proteinuria in human glomerulopathies. *Kidney Int* 66: 1901–1906, 2004
45. Branten AJ, van den Born J, Jansen JL, Assmann KJ, Wetzels JF: Familial nephropathy differing from minimal change nephropathy and focal glomerulosclerosis. *Kidney Int* 59: 693–701, 2001
46. Good KS, O'Brien K, Schulman G, Kerjaschki D, Fogo AB: Unexplained nephrotic-range proteinuria in a 38-year-old man: A case of "no change disease." *Am J Kidney Dis* 43: 933–938, 2004
47. Seefeldt T, Bohman SO, Jorgen H, Gundersen HJ, Maunsbach AB, Petersen VP, Olsen S: Quantitative relationship between glomerular foot process width and proteinuria in glomerulonephritis. *Lab Invest* 44: 541–546, 1981
48. Pirani CL, Pollak VE, Lannigan R, Folli G: The renal glomerular lesions of pre-eclampsia: Electron microscopic studies. *Am J Obstet Gynecol* 87: 1047–1070, 1963
49. Karumanchi SA, Maynard SE, Stillman IE, Epstein FH, Sukhatme VP: Preeclampsia: A renal perspective. *Kidney Int* 67: 2101–2113, 2005