Review Article

New insights into the molecular biology of the glomerular filtration barrier and associated disease

VICKI LEVIDIOTIS and DAVID A POWER

Austin Research Institute, Department of Nephrology, and University of Melbourne, Department of Medicine, Austin Health, Melbourne, Victoria, Australia

SUMMARY: The glomerular filtration barrier of the kidney can no longer be considered as an inert and adynamic structure, viewed by electron microscopy. Molecular biology, medical genetics and protein chemistry have enabled us to further understand the complex structure and function of this highly specialized barrier of the kidney. Minor aberrations of physiology can lead to fatal disease. Recent advances in the understanding of the physiology of endothelial cells, glomerular epithelial cells and the glomerular basement membrane and its components, and how these relate to disease, will be considered systematically.

KEY WORDS: endothelial cells, glomerular basement membrane, molecular biology, podocytes, proteinuria.

INTRODUCTION

The filtration barrier of the kidney consists of three specialized layers that each contribute to its permselective properties; the capillary endothelium, the glomerular basement membrane (GBM) and the single-celled layer of glomerular epithelial cells (GEC) or podocytes.

It is well established that the glomerular filtration barrier behaves as a size-selective filter that restricts the passage of plasma macromolecules based on their size, shape and charge. Anionically charged molecules are filtered in smaller amounts than neutrally charged molecules of comparable size. In contrast, the movement of cationically charged molecules is facilitated.1,2

The exact location of the filtration functions of the barrier has been a matter of debate. Historically, the barrier has been considered to reside in the GBM. More recently, the role of the podocyte and slit-pore diaphragm, in particular, has been appreciated.

Advances in the understanding of the molecular composition of the filtration barrier, over the last decade, have facilitated our understanding of proteinuria. These advances will be reviewed, systematically.

ENDOTHELIAL LAYER

The capillary endothelial layer of the glomerulus is derived from the mesenchyme and is composed of a fine capillary network that branches from the afferent arteriole. It is characterized by the presence of pores or fenestrae that are 70–100 nm in diameter in man.1–5 The endothelial cell nucleus lies adjacent to the mesangium. A network of intermediate filaments and microtubules is present within cells and an extensive network of microfilaments surrounds the endothelial fenestrae. The surface of endothelial cells is highly negatively charged due to the presence of podocalyxin,6 a polyanionic sialoprotein, also found on GEC.7 Endothelial cells form the initial barrier to filtration but their contribution to selective filtration is not marked.

GLOMERULAR BASEMENT MEMBRANE

Interposed between the endothelial layer and the GEC is the GBM. It is composed of three layers identified by electron density. These layers are named the lamina rara interna, lamina densa and lamina rara externa. It is described as a dynamic gel-like acellular meshwork of glycoproteins and proteoglycans. The GBM is thought to play a role not only in filtration and compartmentalization but also in cell adhesion, migration and differentiation.
The major components of basement membranes are laminin, collagen IV, enactin/nidogen and sulphated proteoglycans.

Laminins

Laminins are a family of heterotrimers that are still being described. The three chains that make up the laminins are designated α, β and γ. They form cruciform or Y-shaped heterotrimers. There are currently 12 reported types of laminins assembled from five α, three β and three γ chains. Laminin-11 (α5β2γ1) is a highly restricted trimer found in the GBM and arteriolar basement membrane of the kidney. Targeted mutations of the laminin β2 chain gene results in the development of massive proteinuria at 7 days after birth in mice. The GBM appears normal, ultrastructurally, but the podocyte foot processes are fused. Laminsins are therefore important contributors to the integrity of the GBM.

Collagen type IV

Collagen IV is another ubiquitous component of the GBM. It has been likened to a chickenwire mesh and is composed of 180-kDa α-chains. There are six genetically distinct α-chains (α1–α6) all of which have similar structures. Simplistically, they comprise a short non-collagenous NH2-terminal domain called 7S, a long central collagenous domain composed of interrupted Gly-X-Y amino-acid triplet repeats, and a non-collagenous COOH-terminal domain named NCI. The α-chains assemble to form triple helical rod-like trimers, and in turn these trimers interact to form a chickenwire-like mesh. In the GBM, the predominant α-chains found are α3, α4 and α5.

In man, mutations of the collagen type IV gene result in Alport’s syndrome. As a consequence of these mutations, α1 or α2(IV) collagen chains are substituted to form the GBM. Phenotypically, the GBM appears normal early in life, but it becomes thickened and split over time and ultimately end-stage renal failure develops. Inheritance of this disease in 80% of cases is x-linked. It has been postulated that the abnormal Alport GBM is more susceptible to endogenous protease damage than its normal counterpart. The collagen α5(IV) chain is also of interest because it acts as the antigen in Goodpastures syndrome observed in kidney-transplanted patients, affected by x-linked Alport syndrome.

Recently, GBM collagen IV was identified as a ligand for the receptor kinase discoidin domain receptor 1 (DDR1). Knockout studies revealed that mice develop proteinuria while preserving their renal function, and microelectrophoresis of urine identified albumin, haptoglobin and immunoglobulin peaks. Electron microscopy (EM) studies have identified localized isodense areas of GBM thickening and focal areas of slit diaphragm loss associated with these bulges. These data further confirm the importance of collagen not only as a structural protein but also as a dynamic matrix interacting with its milieu.

Integrins

Integrins are heterodimers that are composed of a single α- and β-unit. Integrins are classified according to the type of β-subunit. The αvβ3-integrin is the major extracellular matrix receptor expressed by podocytes. Endothelial cells express αvβ1, αvβ3, αβ, β complexes and a variety of α-containing integrin complexes. The roles of integrins include cell–cell interactions, interactions between cells and the extracellular matrix, and maintenance of normal tissue architecture. The importance of integrins is appreciated by studying knockout models. αβ-integrin knockout mice develop a syndrome akin to congenital nephrotic syndrome. Integrins are also upregulated in models of crescentic glomerulonephritis where their role as adhesion molecules may contribute to disease. Recently, the interplay between integrins and cathepsins was investigated using knockout studies. Essentially, podocyte migration (or effacement) is reliant upon both these factors being present. If either one is absent then podocyte migration is significantly reduced, suggesting integrins are involved in signalling.

Enactin/nidogen

Enactin and nidogen are different names for the same compound. This glycoprotein is approximately 150 kDa (1217 amino acids) in size and consists of three globular domains separated by two linear segments. It has been likened to a dumb-bell in shape. It is highly susceptible to proteolysis and has both matrix and cell binding properties.

Enactin/nidogen binds tightly to laminin via the γ1 chain. Collagen IV also binds to enactin/nidogen. These interactions occur via the carboxyl-terminal globule of enactin/nidogen. As yet, it is not clear if this compound has a role other than as a structural stabilizer in the GBM.

Proteoglycans

Proteoglycans are found in all basement membranes. They are a class of molecules characterized by a protein
core covalently linked to at least one glycosaminoglycan side-chain. The functions of proteoglycans are due to their glycosaminoglycan side-chains. Glycosaminoglycans are unbranched anionic polymers of either glucose–glucose- or galactose–glucose-derived disaccharides. Heparan sulphates are complex structures belonging to the glycosaminoglycan family. They consist of alternating hexuronic acid and glucosamine residues modified at various positions by sulphation, epimerization or N-acetylation.

Proteoglycans are expressed on the surface of most cell types and are important in cell adhesion to the extracellular matrix, cell–cell interactions, and in the interactions of soluble growth factors with their cell surface receptors. Heparan sulphates bound to structural proteoglycans also bind growth factors and other cytokines. Growth factors including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblast growth factors (FGF-1 and FGF-2) and vascular endothelial growth factor (VEGF) are bound by heparan sulphate proteoglycans (HSPG). The importance of HSPG-bound growth factors is exemplified by VEGF knockout studies. Knockout studies are embryonic lethal, confirming VEGF is vital for normal kidney and vessel development. Vascular endothelial growth factor and VEGF receptors (VEGF-R) are localized to GEC and

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<th>Table 1</th>
<th>Summary of filtration barrier disorders and disease expression</th>
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<td>Layer</td>
<td>Property</td>
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| Endothelium | Fenestrated pores 70–100 nm  
Negatively charged  
Abundant in podocalyxin | ?? |
| GBM constituents | Laminins Heterotrimeric complexes  
α, β and γ chains | β-2 mutation→massive proteinuria |
| Collagen IV | Structural/acts as a DDR1 receptor  
α3–5 mutations→Alport syndrome  
DDR1 KO→proteinuria | α-3 KO develop proteinuria |
| Integrins | Heterodimer complexes  
α and β chains  
structural contributors, ensure cell–cell interactions | ?? |
| Enactin/nidogen | Glycoprotein, 150 kDa, binds to laminin  
Structural role | Glycan-3 alteration→renal dysplasia  
Abnormal HSPG accumulation leads to GBM thickness |
| Proteoglycans | Protein core attached to anionic GAG side-chains  
Cell–Cell interactions | ?? |
| Podocytes | Bind GF (VEGF/FGF/HB-EGF) | Dysregulation of GF binding→proteinuria |
| Inherited abnormalities | NPHS1/nephrin Adhesion molecule/essential for slit pore function | Congenital Finnish nephrotic syndrome, Chr 19  
Autosomal recessive incidence of 1:10 000 |
| | NPHS2/podocin Adhesion molecule/essential for slit pore function | Steroid resistant nephrotic syndrome in children  
Autosomal recessive incidence of 1:10 000  
Chr 1q25–31 abnormality |
| | ACTN-4 α-actinin anchor protein | Variable inheritance→proteinuria  
Chr 19q13 |
| Acquired abnormalities | NEP Podocyte metallocmembrane endopeptidase | Maternal transfer of alloantibodies to NEP→transient neonatal nephrotic syndrome |
| | CD2AP Adhesion molecule, essential for slit pore function | ?? Linked to focal segmental glomerulosclerosis susceptibility |
| | Heparanase β-D-endoglycosidase that degrades anionically charged HSPG | Overexpression→proteinuria  
Inhibition→reduces proteinuria |
| | Cathepsin L Cysteine protease made by injured podocytes | Interacts with α-3 integrin to facilitate podocyte migration |

ACTN-4, actinin-4; CD2AP, CD-2 associated protein; DDR1, discoidin domain receptor 1; FGF, fibroblast growth factor; GBM, glomerular basement membrane; GF, growth factor; HB-EGF, heparin-binding epidermal growth factor; HSPG, heparan sulphate proteoglycans; KO, knockout; NEP, neutral endopeptidases; NPHS, nephrosis homologue; VEGF, vascular endothelial growth factor;
endothelial cells in normal kidney. Furthermore, physiological levels of VEGF ensure normal filtration barrier function: anti-VEGF and VEGF-R antibodies induce reversible proteinuria in animals and in clinical trials. Dysregulation of VEGF expression in the glomerulus also leads to disease. Podocyte-specific over-expression of VEGF resulting in a collapsing nephropathy in contrast heterozygosity is associated with endotheiosis and nephrotic syndrome, a histological lesion akin to pre-eclampsia.

Mutations in genes encoding HSPG are responsible for genetically acquired human diseases including musculoskeletal disorders such as Schwartz–Jampel chondrodystrophic myotonia, dyssegmental dysplasia and hereditary multiple exostosis. Recently, mutations in the HSPG, glypican-3, have been associated with somatic overgrowth and renal dysplasia in man. Knock-out studies of glypican-3 in mice have demonstrated that tight regulation of cell proliferation and apoptosis is critical in the formation of the renal medulla and dependent on this HSPG. Finally, these polyanionic side-chains also confer charge-dependent filtration properties on the GBM and act to stabilize it by binding to laminin, collagen IV and enactin/nidogen.

Abnormal HSPG accumulation is believed to contribute to GBM thickening in diabetes and membranous nephropathy in man. The HSPG alterations, by virtue of charge loss and or masking, have also been proposed to lead to proteinuria. Raats et al. have suggested four mechanisms that may be involved in loss of charge in the GBM.

The first such mechanism is ‘masking of heparan sulphates by immune complexes’. Systemic lupus erythematosus is characterized by the formation of autoantibodies against nuclear antigens. The autoantibody response is T-cell driven and the candidate autoantigen is the nucleosome, which is composed of histones and DNA. The nucleosomes interact directly with the HSPG but the autoantibodies do not bind HSPG. Infusion of nucleosomes results in binding to HSPG followed by antibody attachment. It has been shown that the cationic part of histones binds to the anionic part of HSPG in the GBM. Hence the anionic charge is masked.

The second proposed mechanism is ‘depolymerization of heparan sulphates by radicals’. Systemic renal cells and immunological cells including infiltrating phagocytes, monocytes and macrophages liberate reactive oxygen species (ROS) upon activation. In vitro studies have shown that activated GEC and mesangial cells also liberate ROS. Complement activation, cytokine activation and binding of immune complexes all result in liberation of free radicals. These free radicals are believed to damage proteins, carbohydrates, phospholipids and DNA.

It has been shown that GAG susceptibility to depolymerization by ROS is dependent on the degree of GAG sulphation. In the isolated perfused kidney, ROS activity is known to be high. It has been shown that proteinuria increases in these kidneys, as does heparan sulphate (HS) degradation. The converse occurs with the addition of ROS scavengers such as allopurinol, or a mixture of mannitol/superoxide/catalase. Furthermore, in adriamycin nephropathy, a model associated with marked ROS activity, the addition of dimethyliourea, a hydroxyl radical scavenger, resulted in a reduction of proteinuria and HSPG loss while the agrin core remained unaltered.

These data suggest that HSPG loss is associated with ROS activity. In vitro studies suggest that heparin and hyaluronic acid act as antioxidants and decay molecules. In experimental models of adriamycin nephropathy, murine lupus and streptozotocin-induced diabetes, the administration of heparin has been shown to prevent the loss of HS-associated anionic sites in the GBM, and proteinuria was ameliorated.

The third mechanism is ‘changes in proteoglycan synthesis’. Changed biosynthesis of HSPG may result in abnormal glomerular HS content and/or alterations in the structure or sulphation of HSPG. Such alterations may attenuate charge and alter the anionic properties of the GBM, therefore resulting in proteinuria.

In IDDM, uncharged dextran studies have shown that the GBM has greater porosity as well as a reduction of charge-dependent permeability. Both these traits have been ascribed to a loss of anionic sites in the GBM. Work performed by van Den Born et al. has shown that in both NIDDM and IDDM the HSPG staining intensity is reduced while the protein core remains intact. It has been postulated that this reduction of HSPG staining may occur as a consequence of a reduction in HSPG synthesis rather than its degradation. Furthermore, undersulphation of diabetic HSPG, indicated by lower N- and O- sulphation, contributes to a reduction in the anionic charge of the GBM. It has been postulated that undersulphation occurs as a consequence of a reduction in the activity of the enzyme glucosaminyl N-deacetylase/N-sulfotransferase, the key enzyme in HS sulphation. It is not currently known if hyperglycaemia alters the activity of this enzyme. These abnormalities of HSPG synthesis are found in other BM including the skeletal muscle capillary wall and the dermal–epidermal junction of the skin.

The fourth and final mechanism responsible for HSPG alterations is ‘cleavage by enzymes’. Candidate enzymes include neutral serine proteases and heparan sulphate-specific endoglycosidases, including heparanase.

Polymorph activation results in liberation of neutral serine proteases, including cathepsin G and elastase. Infusion of either of these serine cationic proteases results in them binding to the GBM and proteinuria. Beige mice lacking these proteases, injected with anti-GBM serum, do not develop proteinuria. However, intravenous administration of these proteases results in albuminuria and a reduction in HSPG staining, while...
the protein core remains intact. In vitro studies have shown that elastase binds to the anionic component of the GBM, cleaves agrin near the HS attachment sites and releases HS fragments.\(^6^6\)

Heparanase is a β-D-endoglycosidase that degrades the anionically charged GAG side-chains of HSPG. Loss of these anionic side-chains may result in attenuation of the anionic properties of the GBM and, in turn, a loss of its perselective properties. Other possible sequelae include liberation of HSPG-bound growth factors and finally, alteration of the conformation of the endothelial and epithelial GBM contact points. Recently, a transgenic heparanase over-expression approach resulted in mice developing significant proteinuria and renal dysfunction, and the associated EM appearance of kidneys was consistent with minimal change disease.\(^5^7\) Conversely, heparanase inhibition studies have resulted in a reduction of proteinuria, further confirming the importance of heparanase in the development of proteinuria.\(^5^8,5^9\)

### PODOCYTES

The epithelial cells that rest on the GBM are called podocytes or visceral epithelial cells. They are distinct from the relatively flattened cells that line the Bowman capsule and are the largest cells contained within the glomerulus. Podocytes are unique, possessing a large number of cytoplasmic extensions or foot processes that rest on the lamina rara externa of the GBM. As polarized cells, podocytes provide a permeability barrier between two very different environments.

In contrast to glomerular endothelial cells, podocytes do not typically proliferate in vivo. Normally, after lethal podocyte injury podocyte number is depleted, the basement membrane is denuded and scarring ensues. Over the last decade our understanding of cell cycle regulation has been advanced. In brief, after mitogenesis, cells either enter the G\(_0\) phase of the cycle and become quiescent or re-enter the cycle or G\(_1\) phase. Cyclin-dependent kinases (CDK) are involved in driving DNA synthesis and cell proliferation via phosphorylation of CDK inhibitors. The INK4 family is made up of four proteins: p16\(_{\text{INK4a}}\), p15\(_{\text{INK4b}}\), p18\(_{\text{INK4c}}\) and p19\(_{\text{INK4d}}\), and the Cip/Kip family includes the p21\(_{\text{Cip1}}\), p27\(_{\text{Kip1}}\) and p57\(_{\text{Kip2}}\) proteins.\(^6^0,6^1\) The p27\(_{\text{Kip1}}\) and p57\(_{\text{Kip2}}\) proteins are strongly expressed in mature podocytes; in contrast podocytes during embryogenesis lack these CDK inhibitors. Knockout studies have also been helpful in trying to understand the significance of these inhibitors. Glomerular development appears normal in p27\(_{\text{Kip1}}\) and p57\(_{\text{Kip2}}\) knockouts, but animals with dual genes deleted have larger glomeruli with an increased number of podocytes.\(^6^3\) The assumption is that these genes are important in controlling the final complement of podocytes.

More recently, studies investigating the impact of complement-mediated podocyte injury have confirmed an increase in podocyte cyclins and CDK. However, there is only a limited amount of DNA synthesis suggesting the presence of cell cycle inhibitors; this has been confirmed and both p21\(_{\text{Cip1}}\) and p27\(_{\text{Kip1}}\) are increased subsequent to complement-mediated injury.\(^6^4\) Furthermore, complement-mediated injury also induces DNA damage and ultimately it might be that podocyte proliferation is arrested by direct DNA damage.\(^6^5\)

Idiopathic collapsing glomerulosclerosis and HIV-associated nephropathy are characteristicly associated with podocyte proliferation. Marker studies have confirmed that this proliferative response is associated with a reduction in the p27\(_{\text{Kip1}}\) and p57\(_{\text{Kip2}}\) markers. In vitro studies have also confirmed that HIV induces podocyte cyclin expression and a loss of the differentiation markers synaptopodin and podocalyxin. These changes are associated with podocyte proliferation. In contrast, diseases not characterized by proliferation, including membranous nephropathy, minimal change disease and focal segmental glomerulosclerosis, are not associated with loss of these proliferation inhibitors.\(^6^6\) Finally, podocyte stretch or increases in intraglomerular pressure leads to a reduction of proliferation markers and proliferation, with a concurrent increase in CDK inhibitors.\(^6^7\) A further understanding of cell cycle regulators and their eventual manipulation may result in development of agents promoting repair of injured podocytes.

Podocytes synthesize a number of enzymes including heparanase,\(^6^8\) neutral endopeptidases (NEP) and dipeptidases, which degrade highly biologically active peptides. They also synthesize autacoids, endothelin and growth factors including PDGF, basic fibroblast growth factor (bFGF), VEGF, heparin-binding epidermal growth factor (HB-EGF) and TGF-β.\(^6^9\)

In addition to forming an integral part of the filtration barrier, podocytes are involved in GBM turnover and regulation of the ultrafiltration coefficient and provide support for the glomerular tuft. Podocytes are also responsive to angiotensin II (ANG II),\(^6^9\) inhibition of ANG II with angiotensin-converting enzyme (ACE) inhibitors has been shown to slow the progression of glomerulosclerosis.\(^7^0\) Podocyte hypertrophy is reduced by ACE in a model of subtotal nephrectomy, indicating that ANG II directly influences podocyte morphology and growth.\(^7^1\) These data suggest that angiotensin II is important in the function of podocytes.
Adjacent podocyte foot processes, usually derived from different podocytes, interdigitate. The potential space between foot processes varies from 25 to 60 nm, and is referred to as the filtration slit or slit pore. The junction between podocytes is referred to as an ‘adherens junction’, and is characterized by the presence of Ca²⁺-dependent cell–cell adhesion molecules (cadherins) that are linked to intracellular actin and myosin filaments.

A thin membrane called the slit membrane or slit diaphragm makes up the adherens junction. This membrane is approximately 60 nm from the GBM. Closer inspection of the slit membrane reveals a central 11-nm wide filament, which has filamentous cross-bridges that link the adjacent pedicles. Nephrin has been localized to the slit diaphragm by immunogold-electron microscopy. Mutations of this protein result in proteinuria. In addition, P-cadherin has been localized to the slit diaphragm. Controversy exists as to the importance of P-cadherin, with some investigators believing that it represents the core protein that forms the slit diaphragm. P-cadherin has both an intracellular and extracellular component. It is believed that the intracellular components are connected to β and γ-catenins, which in turn are connected to the actinin cytoskeleton via α-actinin. Zona occludens-1 (ZO-1) belongs to the membrane-associated guanylate kinase (MAGUK) family of proteins and it also makes up the slit diaphragm. Zona occludens-1 is believed to couple extracellular signalling pathways via the intracellular cytoskeleton. It is therefore evident that the intracellular environment is in constant communication with both the extracellular and slit diaphragm milieu.

Podocytes also contain membrane-associated proteins. The principal sialo-protein found on the apico-lateral surface of podocytes is podocalyxin. Podocalyxin is a highly negatively charged protein that is believed to contribute to the permselective properties of the GBM. In addition, the C3b receptor, Heymann nephritis antigen (gp330) and podoplanin (a 43-kDa membrane glycoprotein) are located on the surface of rat podocytes. Podoplanin is believed to contribute to the maintenance of podocyte foot processes and, hence, glomerular permeability. Synaptopodin is a 74-kDa protein found in association with actin and is exclusive to mature podocytes, located in the foot processes. Its postulated role is in podocyte motility and its absence after injury is associated with podocyte drop out.

More recently, NEP, a metalloenzyme endopeptidase, was identified as a target antigen on podocytes and has been implicated in the development of antenatal membranous nephropathy. Elaborate studies have confirmed that mothers with NEP gene mutations develop alloantibodies to the conserved fetal NEP antigen; alloantibody transmission results in the development of a largely self-limited nephrotic process in neonates.

Damage to podocytes is also associated with a loss of the maturity markers WT-1 (a podocyte-specific nuclear protein), GLEPP-1, podocalyxin and the C3b receptor. Loss of protein markers is believed to indicate the extent and irreversibility of podocyte injury.

Many diseases associated with proteinuria are characterized by foot-process effacement (FPE) or fusion. Foot-process effacement is associated with stereotypical structural alterations regardless of the inciting injury. It is a process in which the foot processes of two adjacent podocytes retract in a coordinated fashion. Consequently, the interdigitating pattern of the podocytes and podocyte cytoskeleton are altered and the slit membrane is reduced. Similar changes have been observed in rodent kidney after infusion of protamine sulphate, a polycationic substance that interacts with the anionic sites of the GBM. Infusion of neuraminidase, in contrast, removes sialic acid and causes a detachment of endothelial cells and epithelial cells. These data suggest that the anionic changes of these cells are important in the maintenance of the normal structure and function of the filtration barrier.

Closer examination of podocytes reveals a well-developed Golgi and lysosomal system. In addition, an extensive microtubular, microfilament and intermediate filament system is present in the cytoplasm and an actin-based filamentous cytoskeleton can be observed in foot processes. Actin, myosin, α-actinin and vinculin form bundles that run parallel to foot processes and connect to the microtubular cytoskeleton. These actin bundles are linked to the slit membrane by the adapter protein ZO-1, catenins and the 80-kDa CD-2 associated protein (CD2AP). The importance of CD2AP was recently highlighted by knockout studies: animals developed congenital nephrotic syndrome and FPE, and died by 6 weeks from renal failure. The CD2APα–animals, in contrast, developed less severe disease by 9 months of age with a histological pattern similar to idiopathic focal segmental glomerulosclerosis. EM studies have revealed widespread immune deposits, and defective clearance has been speculated. Normally, CD2AP is podocyte associated and is located at the cytoplasmic face of the slit diaphragm. It also acts as a T-cell adhesion protein but the immune function of CD2APα–animals remains unaffected. Recently, heterozygous CD2AP mutations were detected in two out of 30 patients with focal segmental glomerulosclerosis; 45 control samples contained a normal amount of CD2AP. As yet, it remains hypothetical that heterozygous mutations may increase glomerulonephritis susceptibility.

Actin bundles are also linked to the GBM by integrins and dystroglycans. It is believed that signalling to the podocytes can occur from the GBM. This so-called ‘outside-in communication’ has been proposed to influence podocyte cytoskeletal changes. Administration of protamine results in loss of actin bundles, cell shape alterations, and a reduction and redistribution of focal contact points.
Clearly, podocytes are a vital component of the filtration barrier. They not only conceal the underlying GBM but also contribute to the anionic properties of the filtration barrier by virtue of the proteins associated with their cell membrane. Podocytes also communicate with each other via a complex intracellular microfilament network that attaches to a variety of linker proteins.

**INHERITED PODOCYTE ABNORMALITIES**

Two new proteins located at the slit diaphragm have been identified. These are nephrin and podocin, the NPHS1 and NPHS2 gene products, respectively.

Mutations in nephrin result in the development of the congenital Finnish nephrotic syndrome, inherited as an autosomal recessive trait with an incidence of 1:10,000 births in Finland. The lesion characteristic of this defect is associated with diffuse FPE. Nephrin is 136 kDa in size and is located on chromosome 19, it has been described as an adhesion molecule belonging to the immunoglobulin family and has an intracellular and large extracellular domain. It is postulated that nephrin proteins from the slit diaphragm assemble to form an isoporuous filter in conjunction with ZO-1, P-cadherin and the catenins. Any disruption of this isoporuous filter is associated with podocyte foot process effacement and is associated with diffuse FPE. Nephrin is 136 kDa in size and located on chromosome 19q13. It has been described as an adhesion molecule belonging to the immunoglobulin family and has an intracellular and large extracellular domain. It is postulated that nephrin proteins from adjacent glomerular epithelial cells to form an isoporuous filter in conjunction with ZO-1, P-cadherin and the catenins. Any disruption of this isoporuous filter is associated with podocyte foot process effacement and podocyte foot process effacement.

Malignant polyoma virus infection is known to be associated with the development of proteinuria. Podocin abnormalities have been linked to X-linked Alport syndrome. A recent study has shown that podocin is associated with a reduction in podocin and CD2AP, suggesting that the interplay of these three molecules is essential for maintenance of the filtration barrier. In humans, nephrin expression is reduced in minimal change and membranous nephropathy. Nephrin is an adhesion molecule belonging to the immunoglobulin family and has an intracellular and large extracellular domain. It is postulated that nephrin proteins from adjacent glomerular epithelial cells to form an isoporuous filter in conjunction with ZO-1, P-cadherin and the catenins. Any disruption of this isoporuous filter is associated with podocyte foot process effacement.

Podocin, the product of NPHS2, also localizes to the slit membrane and its absence is associated with steroid-resistant autosomal recessive nephrotic syndrome in children. Podocin abnormalities have been linked to chromosome 1q25-31 and the gene product is 42 kDa. Like nephrin it, too, is exclusively expressed by podocytes in the glomerulus. Anti-podocin antibodies after transplantation may be responsible for disease recurrence in this group, but this has not been verified.

Mutations of the ACTN4 gene and hence gene product, α-actinin, result in familial focal segmental glomerulosclerosis. This disorder is inherited as an autosomal dominant disease with variable penetrance and occasionally in an autosomal recessive manner; the chromosomal abnormality has been identified to chromosome 19q13. The absence of the anchor protein, α-actinin, is believed to significantly alter podocyte foot process integrity, therefore resulting in proteinuria. The aforementioned data confirm that the slit diaphragm is emerging as an important contributor to the induction of proteinuria.

**CONCLUSION**

A better understanding of the molecular composition and physiology of the filtration barrier has therefore enabled us to further understand the disease processes of this permselective barrier. The importance of these components is exemplified by disease expression in their absence (Table 1). Ongoing research is required to advance our knowledge and direct therapies.

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