Proteinuria and function loss in native and transplanted kidneys

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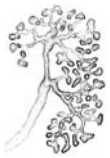

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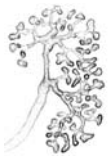

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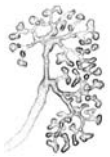
"Bones can break, muscles can atrophy, glands can loaf, even the brain can go to sleep, without immediately endangering our survival, but when the kidneys fail to manufacture the proper kind of blood neither bone, muscle, gland nor brain can carry on".

Homer Smith, From Fish to Philosopher (1).

It is probably of limited value to ascribe the kidney a function superior to that of other organs; this quote merely indicates the importance of the kidney for the maintenance of the harmonious composition of body fluids – a function the kidney performs in a way that joins efficacy with elegance. Some of these processes will be lined out in this introductory chapter. The first section gives an overview of kidney development, anatomy and function. This is followed by a description of malfunction of the kidney and its consequences in the second part, which focuses on the development of proteinuria. Complete failure of the kidneys necessitates kidney replacement therapy or transplantation. The third section gives an overview of long-term problems that limit the success of transplantation as a treatment modality. This is followed by an outline of the thesis.



PART 1 THE KIDNEY: DEVELOPMENT, ANATOMY AND FUNCTION

Overview of kidney anatomy and function

The kidney is a bean-shaped organ with a length of about 11 cm and a weight of approximately 150 grams. The kidneys are located in the retroperitoneal space at either side of the vertebral column, just below the diaphragm. The concave part of the kidney forms the renal sinus that contains the renal vessels, nerves, and the renal pelvis. The arterial blood supply sprouts directly from the aorta; the renal veins drain into the vena cava. Via the ureter the renal pelvis is continuous with the bladder and the outside world. The functional tissue of the kidneys surrounds the renal sinus, and is divided into cortex and medulla.

The human kidney contains about one million functional units or nephrons (30,000 in rats). A nephron consists of a filtration body – a tuft of anastomosing capillaries called glomerulus – connected to a long, tortuous tubule. The glomerular capillaries function as a microfilter that restricts passage of blood cells and proteins, but is permeable for smaller plasma components and waste products dissolved in the plasma water. Some ten percent of the blood that flows into the glomerulus is filtered, and the ultrafiltrate is delivered into the tubule (see below for a more comprehensive description of glomerular filtration). The blood that escapes filtration flows into the capillary network in the tubular compartment. Here, the tubular epithelial cells exchange substances between the fluids in the tubular lumen and the blood, thus gradually converting the ultrafiltrate into urine. Most of the exchange is from tubular lumen to the blood: the tubular epithelial cells reabsorb almost all water filtered in the glomerulus, together with salts, glucose, amino acids, vitamins, and other small molecules. But there is also active excretion of substances from the blood into the tubular lumen. When the urine via the collecting ducts flows into the renal pelvis, it is a concentrated solution of dissonants that the nephron has produced to maintain the harmonious composition of the blood.

In the next paragraphs, the microscopic anatomy of the nephron is described in some more detail, along with an overview of glomerular filtration. First, a brief overview of kidney development is provided.

Development

Pathophysiologic processes sometimes recapitulate mechanisms that play a role in embryonic development (2). It is therefore of importance to understand some of the processes that take place during the organogenesis of the kidney. This paragraph describes the development of the kidney, with a focus on the development of the glomerulus. For more extensive overview of the developmental processes and their molecular regulation the reader is referred to several reviews on the topic (3-7).

The kidneys develop from the intermediate mesoderm of the embryo. The intermediate mesoderm is made up of two tissue compartments, the nephric duct and the nephrogenic cord, that form the different structures of the kidney. Derivates of the nephric duct form the collecting system of the kidney. The glomerular and tubular parts of the nephron arise from the nephrogenic cord.

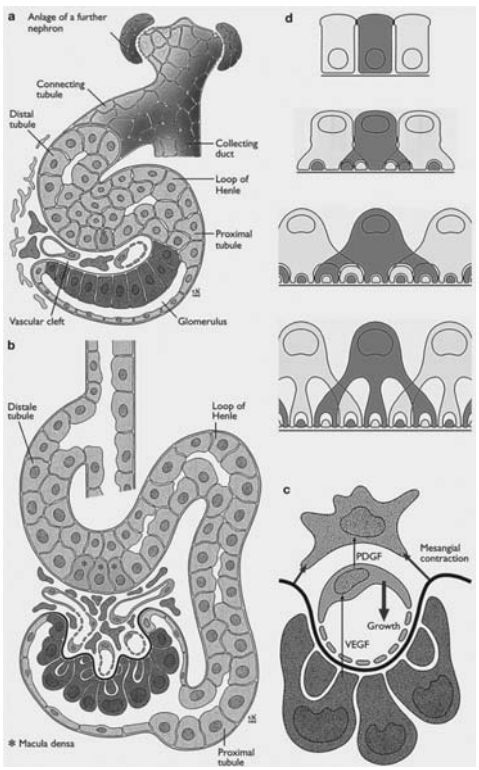
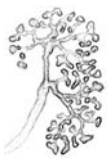


Figure 1. Embryonic development of the glomerulus. In the S-shaped body, the presumptive podocytes form a columnar epithelium that is continuous with the tubular epithelial cells. Endothelial and mesangial cells migrate into the vascular cleft (a). In the capillary loop stage, further differentiation of podocytes takes place (b). The presumptive podocytes start to form foot processes that interdigitate with those of neighboring cells (d). Differentiation of podocytes stimulates further development of endothelial and mesangial cells (c). See text for further details. Adapted from (152), with kind permission from S. Karger AG and the author.

During embryonic development, a sequential development of three more or less separate excretory systems, referred to as the pro-, meso-, and metanephros, takes place through interaction between the nephrogenic cord and the nephric duct. The pronephros is formed first and most cranial. It is a transient, non-functional structure that consists of a few tubules that connect to the nephric duct. Caudal to the pronephros the nephric duct induces the formation of the mesonephros, a structure consisting of a glomerulus and a relatively simple tubule. Though also transient, the mesonephron forms a functional excretory organ (8). The blood supply to each glomerulus sprouts directly from the aorta, while the tubules drain into the nephric duct that at this stage of development is called the mesonephric or Wolffian duct.

Subsequent to the development of the mesonephros, and again more caudally, the



development of the permanent kidney or metanephros starts. The metanephros develops through reciprocal interactions between the mesonephric duct and a condensed part of mesenchyme referred to as the metanephric blastema. The metanephric blastema induces the formation of a bud from the mesonephric duct. This ureteric bud grows into the metanephric blastema, and induces it to form a cap on the ureteric bud. The mesenchymal cap in turn induces dichotomous branching of the ureteric bud. The close apposition of the tips of the ureteric bud and the proliferating mesenchyme leads to continuous branching of the ureteric bud (a movie of ureteric bud branching will play in the upper right corner when quickly thumbing through the pages of this book from back to front. Courtesy of dr. Frank Constantini, Columbia University Medical Center). This reciprocal stimulation eventually produces an arborized system of ducts, of which each final branch is in close proximity to a mesenchymal condensate. Remodeling, ie, coalescence of the branched ducts shapes a system of collecting ducts that via the renal calyces, renal pelvis and ureter drains into the bladder; the mesenchymal condensate undergoes a sequence of events that transforms it into a functional nephron that connects to the collecting duct.

The invasion of the ureteric bud into the metanephric mesenchyme rescues the mesenchymal cells from apoptosis, and induces expression of the genes paired box 2 (PAX2) and Wilms Tumor 1 (WT1). In the presence of PAX2 and WT1 expression, and through stimulation by Wnt-4 and Bone morphogenetic protein 7 (BMP-7), mesenchymal cells acquire an epithelial phenotype, a process called mesenchymal-to-epithelial transition (MET) (9-12). After completing MET, the condensed cells start to form a vesicle. The renal vesicle then undergoes a series of morphologic changes: it first changes into a comma-shaped body that then elongates and folds back on itself to form an S-shape (figure 1a). The molecular mechanisms that regulate this process are not completely known. From this stage onward, epithelia in different parts of the S-shaped body start to differentiate, thus forming the at least 15 different epithelial cell lineages of the future nephron (5). During differentiation, expression of PAX2 is downregulated, probably through increasing expression of WT1 (13). This occurs most prominently in the proximal part of the S-shaped body, where the future podocytes show high expression of the WT1 protein, which continues to be a marker of podocytes throughout differentiation and in the mature glomerulus (14,15). The presumptive podocytes that surround the capillary tuft are first organized as a columnar epithelium, but progressively lose their lateral cell-cell adhesions and begin to form cellular extensions called foot processes. Eventually, the cell bodies of the podocytes float freely in the urinary space, and the adhesion between two adjacent podocytes is restricted to the slit diaphragm between the interdigitating foot processes (figure 1d) (16,17). Exactly which signals drive foot process formation is currently unknown, although the interaction with the GBM seems to be of crucial importance (17).

In the S-shaped stage of glomerular development, the presumptive podocytes express vascular endothelial growth factor A (VEGF-A) (18). This, either through stimulation of local angioblasts or

through incorporation of endothelial cells sprouting from nearby vessels (19,20), leads to the recruitment of endothelial cells into the cleft of the S-shaped body adjacent to the future podocytes (figure 1a,b) (14,21). In turn, endothelial cells of the capillaries express platelet-derived growth factor-B (PDGF-B), thus recruiting mesangial cells – of which the origin remains elusive (22) – that express PDGF receptor-β (figure 1c) (23). Through further branching of capillaries, differentiation of epithelial cells, and deposition of extracellular matrix, the three glomerular cell types shape the glomerulus (17,23).

The tubules elongate and differentiate and the most distal part of the S-shaped body connects with the branch of the ureteric bud that has developed into the collecting duct. By week 34 of fetal development, the formation of nephrons is complete. Further differentiation of the renal tissues continues postnatally (24).

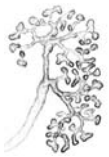
The glomerulus

As the renal artery enters the kidney, it subdivides in smaller arteries and arterioles. The last branch, the afferent arteriole, gives rise to a tuft of anastomosing capillaries called the glomerulus. Like all capillaries, the glomerular capillary lumen is lined by endothelial cells. At the inside, the tuft of capillaries is held together by mesangial cells, while the outer aspect of the capillaries is covered by the visceral epithelial cells or podocytes. The glomerulus is surrounded by Bowman's capsule and its parietal epithelial cells. Bounded by the visceral and parietal epithelial cells is the urinary space, which is continuous with the lumen of the tubuli. The different glomerular cell types will be discussed, followed by an overview of podocyte cell biology and glomerular filtration.

Glomerular cell types

The endocapillary lumen is lined with endothelial cells that are fenestrated. The cells rest on the glomerular basement membrane (GBM). With special staining techniques, a thick layer of glycocalyx has been visualized on glomerular endothelial cells, a finding that may shed light on the contribution of endothelial cells to glomerular filtration (25-27).

Mesangial cells are located between the glomerular capillaries, and reinforce the structure of the glomerulus. Contractile extensions of the mesangial cells bridge opposing portions of the GBM, and balance the outward forces of the blood pressure (28). Additionally, contraction of the mesangial cell myosin filaments provides a mechanism for control of glomerular blood flow. In this regard, mesangial cells resemble smooth muscle cells. Mesangial cells also have macrophage-like



functions, through which they are able to clear macromolecules and immune complexes from the glomerulus.

The outer aspect of the GBM is covered by visceral epithelial cells, or podocytes. These are highly differentiated cells with a complex, arborized phenotype. The podocyte cell body floats in the urinary space and gives rise to primary processes. The podocyte attaches to the GBM by means of foot processes, further cellular extensions that sprout perpendicularly from the major processes. Foot processes of two adjacent primary processes interdigitate, leaving a 40 nm space or 'slit pore' between them that is bridged by the slit diaphragm. In this way, the podocyte foot processes and the interposed slit diaphragm completely enwrap the glomerular capillaries. Recent three-dimensional ultrastructural analysis of the podocyte revealed an even further complexity. The space between the foot processes and the overlying cell body and primary processes appeared to be more restricted than previously appreciated, delineating a so-called sub-podocyte space. The finding that this space covers as much as 60 percent of the glomerular surface may bring important insights into the mechanisms of glomerular filtration (29,30).

Apart from its role in glomerular filtration, the podocyte probably also functions as a structure-stabilizing cell, providing forces to counteract capillary distension (31). Additionally, podocytes produce the extracellular matrix components that make up the GBM, and they provide growth factors like TGF-β and VEGF. The latter plays a role in the maintenance of the glomerular endothelium. The cell biology of the podocyte will be discussed in more detail in the next paragraph.

In contrast to the elaborately shaped podocytes and not reminiscent of their common embryonic origin, parietal epithelial cells (PECs) appear as a simple flat epithelium that lines Bowman's capsule. Recent reports have made clear that occasionally podocyte-like cell types can be found at parietal cell positions (32). These cells may be PECs that transdifferentiate to become podocytes (33). PECs themselves have long been regarded as relatively inert cells that, although they may show secondary reactivity in response to glomerular pathology, play no crucial role in glomerular diseases. Recent studies have changed this view (34). Several reports have indicated that PECs do play a role in animal models of focal segmental glomerulosclerosis (35,36) and HIV-associated nephropathy (37), as well as in human glomerular diseases (38,39).

Glomerular extracellular matrix

During the development of the glomerulus, endothelial cells and podocytes together produce the extracellular matrix they rest on and that separates the two cell types: the 300 nm thick GBM. In the mature glomerulus, all three glomerular cell types synthesize components of the GBM (40-42)

Ultrastructurally, the GBM consists of three layers: the laminae rara interna, densa and rara externa, but it is not known whether these different layers also represent different molecular compositions, or are a reflection of a fixation artifact (43). The molecular constituents of the GBM include

laminin (isoform $\alpha5\beta2\gamma1$), collagen IV (isoform $\alpha3\alpha4\alpha5$), heparan sulphate proteoglycans agrin, perlecan, collagen XVIII, syndecan, fibronectin, and nidogen/entactin. The collagen IV and laminin chains are crosslinked by nidogen, and form a strong, porous, network. The GBM proteoglycans are responsible for the negative charge of the GBM, thought to be instrumental in glomerular ultrafiltration (see below).

Mesangial cell extensions and mesangial ECM fibers are continuous with the GBM, and are thought to reinforce the glomerular structure (44). The mesangial matrix is composed of fibronectin, collagen IV (isoform $\alpha1\alpha1\alpha2$), laminins (isoform $\alpha5\beta1\gamma1$) and proteoglycans, with – similar to the GBM – heparansulphate as the major glycosaminoglycan (45). Many glomerular diseases result from an imbalance between mesangial matrix synthesis and degradation. For example, expansion of mesangial matrix is an important feature of diabetic nephropathy.

Cell biology of the podocyte

The molecular processes that support the podocyte's complex architecture and provide the basis for its function have received much attention in recent years. The elucidation of genetic causes of rare hereditary disorders, the generation of conditional and inducible knock-out mice, and the possibility to culture human and rodent podocytes have been instrumental in the advancement of understanding the cell biology of the podocyte.

Cytoskeleton

An elaborate cytoskeleton that combines strength with flexibility is indispensable for the correct shape and function of the podocyte. In the foot processes all membrane domains are physically linked to the cytoskeleton, and at the functional level the establishment of a connection between membrane associated molecules and the cytoskeleton has become a common theme.

The cytoskeleton of the cell body and primary processes is composed of microtubules and intermediate sized filaments such as vimentin. Foot processes lack these filament types; their cytoskeleton instead consists of actin filaments. Ichimura et al showed that there are two distinct populations of these actin filaments in the foot processes: a dense bundle that runs along the longitudinal axis of the foot processes, and a cortical actin network beneath the plasma membrane (46). At the base of the foot processes the bundle of actin filaments connects to that of the adjacent foot process as well as to the cytoskeleton of the major processes (figure 2). At the tip of the foot process the filaments are attached to the GBM via various linker molecules. Apart from actin, the foot process cytoskeleton contains several actin-associated molecules, including myosin and α-actinin-4. This suggests that the cytoskeleton has contractile capacities, and may serve to counteract the capillary pressures (14,31,47), although such contractions have until now not been observed in vivo (48).

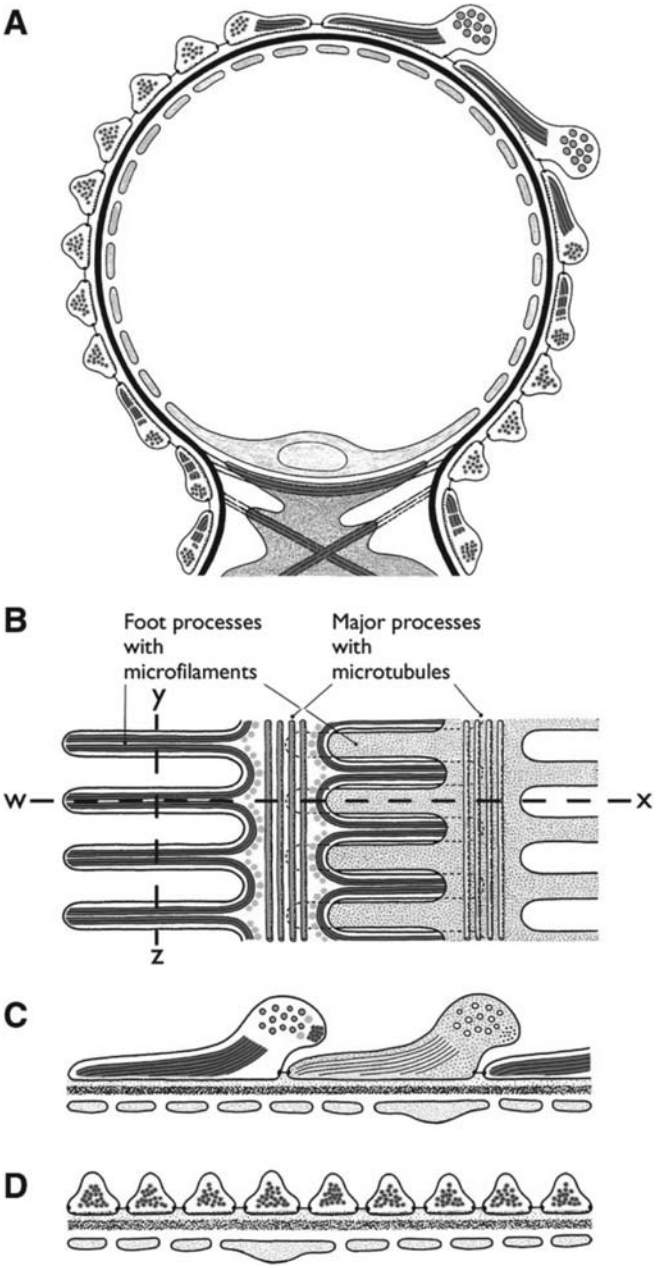


Figure 2. Organization of the podocyte cytoskeleton. A cross section through a capillary loop shows that the capillary is surrounded by podocyte foot processes. At the base of the capillary, contractile mesangial cell filaments are attached (a). A schematic view from above shows that the foot processes sprout perpendicular to the major processes. The cytoskeleton of the major processes is composed of microtubuli, to which the actin-based cytoskeleton of the foot processes is attached (b). In (c) and (d) the lateral view capillary wall is depicted, corresponding to the w-x and y-z line in (b), respectively. Adapted from (14), used with permission from The American Physiological Society and the author.

In response to damage, the podocyte ultrastructure and organization of the actin cytoskeleton are severely altered: foot processes are typically lost – a process called foot process effacement – and the highly organized actin fibers are rearranged to form a dense mat of interconnected fibers at the base of the effaced foot processes (14,49,50). This extensive and sometimes quickly reversible rearrangement of cytoskeletal elements has stimulated research into the regulation of actin and actin associated proteins in the podocyte.

Actin filament elongation is regulated by Rho GTPases such as RhoA, rac, and cdc42, and these molecules probably have an important role in the formation of the cytoskeleton of the podocyte (51). Indeed, mice that lack an inhibitor of Rho GTPases, ie, in which these proteins are more active, develop heavy proteinuria and foot process effacement (52). Others have shown that the deleterious effect that plasma

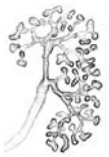
proteins have on podocytes is regulated in a RhoA dependent manner (53). Other actin regulating molecules including cortactin, Ena/VASP, and Arp2/3 are expressed in podocytes, illustrating the wide range of actin cytoskeleton activity displayed by podocytes (48).

The crucial role of the actin cytoskeleton is underscored by the fact that dysregulation of actin associated molecules results in a loss of adequate glomerular function. For example, MYH9, the gene coding for myosin heavy chain IIA, is related to the development of Fechtner syndrome that includes podocyte abnormalities (54). An upregulation of α-actinin-4 was shown to precede proteinuria and development of foot process effacement in PAN nephrosis (55). Later, mutations in ACTN4, the gene coding for α-actinin-4, were shown to be the cause of a hereditary form of late-onset focal segmental glomerulosclerosis (56). These mutations lead to an increased affinity of α-actinin-4 for actin, probably reducing cytoskeletal dynamics (56). At the same time, these mutations increase the protein degradation rate (57), and mice that lack α-actinin-4 also have glomerular disease (58), showing that both gain- and loss-of-function mutations in ACTN4 impair correct podocyte function.

Heat shock protein 27 has been reported to play a role in actin polymerization, and has been implicated in foot process effacement (59-61). Synaptopodin, also called pp44, was identified by Mundel et al to be an actin binding protein which expression is restricted to podocytes and neurons (62,63). Disruption of synaptopodin does not lead to glomerular disease, but seems to lower the threshold for development of glomerular abnormalities (64,65). This may relate to the involvement of synaptopodin in actin dynamics: synaptopodin regulates the actin bundling activity of α-actinin-4 (64) and prevents the proteasomal degradation of RhoA (66).

Apical membrane

Mature podocytes are polarized cells, and the apical membrane of the foot processes is fundamentally different from the basal and baso-lateral parts (see figure 3 for a schematic representation of the molecular organization of the podocyte foot process). A long-known characteristic of the apical membrane is that it is highly negatively charged (67). Kerjaschki et al identified the sialomucin podocalyxin as the molecule that provides this negative charge by means of several sialic acid residues (68). It has been suggested that the extracellular domain of podocalyxin serves as a 'spacer molecule' with anti-adhesive characteristics, preventing a connection between two adjacent foot processes (69,70). In keeping with the importance of podocalyxin for the integrity of the podocyte, podocalyxin knock-out mice show an impaired kidney development, with failure of podocytes to form foot processes (71). In vivo, interference with podocalyxin, for example through infusion of the polycation protamine sulfate, leads to proteinuria and foot process effacement (72). Such a change in foot process architecture would require a reorganization of the actin cytoskeleton, suggesting that podocalyxin interacts with this structure. Indeed, Farquhar's group showed that podocalyxin is physically linked to actin via a complex including NHERF2



and ezrin, and that this linkage is disrupted in foot process effacement (72). Follow-up studies showed that podocalyxin may also directly bind ezrin and activate RhoA, showing additional actin-modulating properties (73).

Recently, the heavily glycosilated negatively charged α-subunit of dystroglycan has been suggested to perform a function similar to that of podocalyxin (74). Loss of dystroglycan from the apical membrane may result in foot process effacement, in addition to a pathogenic role that loss of dystroglycan from the basal site of the foot process may have (discussed below) (75).

The transmembrane glycoprotein podoplanin adds further negative charge to the apical membrane (76-78). Little is known about the intracellular connections of podoplanin in the podocyte, although in other cell types this protein has been shown to interact with ezrin (79,80).

GLEPP-1 (protein tyrosine phosphatase receptor type O) is a phosphatase expressed in the apical membrane of the podocyte. As demonstrated in knock-out mice, the protein has a role in the correct shaping of the actin cytoskeleton (81), but the substrates of its phosphatase function remain undefined (14).

Slit diaphragm region

The slit diaphragm that spans the space between two adjacent foot processes is inserted in the basolateral membrane of the foot process. The multitude of proteins and protein-lipid complexes that make up and support the slit diaphragm and both physically and functionally link the structure to other parts of the cell, make this a highly specialized cell compartment.

Molecular architecture of the slit diaphragm

Although the slit diaphragm and some of its morphological characteristics have been recognized since the application of electron microscopy in kidney research (82,83), its molecular constituents have long remained obscure. The tight junction protein zonula occludens-1 (ZO-1) was one of the first molecules described to be associated with the slit diaphragm. Reiser et al showed that P-cadherin and ZO-1 co-localize at the slit diaphragm region, suggesting that the slit diaphragm is a modified adherens junction (84).

In 1988, Shimizu's group showed that the injection of monoclonal antibody raised against a component of rat glomeruli (mAb 5-1-6) in rats produced massive and transient proteinuria. The epitope recognized by this antibody localized almost exclusively to the slit diaphragm (85). Proteinuria, induced through infusion of the antibody or through protamine sulfate, caused an apical dislocation and internalization of the protein (86,87).

A breakthrough in podocyte biology was the finding that mutations in NPHS1, coding for nephrin, cause the congenital nephrotic syndrome of the Finnish type (CNF) (88), a syndrome characterized by proteinuria in utero and rapid development of end-stage renal disease requiring kidney transplantation (89). Nephrin was found to have a restricted expression, and in the kidney

localized to the slit diaphragm (90-92). In retrospect, the epitope recognized by mAb 5-1-6 appeared to be identical to rat nephrin (93). Because nephrin, a member of the Ig protein superfamily, has a large extracellular domain and could form homodimers in vitro (94), it was suggested that nephrin strands from opposing foot processes may form the actual slit diaphragm through nephrin-nephrin interactions (95). This hypothesis was tested in an extensive study using electron tomography in combination with immuno-gold labeling of nephrin in glomeruli from various mammalian species, both in health and disease (96). From this study, it was concluded that nephrin, together with other proteins that were not molecularly identified, contributes to the slit diaphragm. In CNF patients the slit pore was much smaller and did not contain a slit diaphragm. Confirming these findings, different reports on nephrin knockout mice consistently showed heavy proteinuria and early death of the mice, with absence of the slit diaphragm (97-99), although foot processes were assembled normally (17). These studies established nephrin as an important structural component of the slit diaphragm.

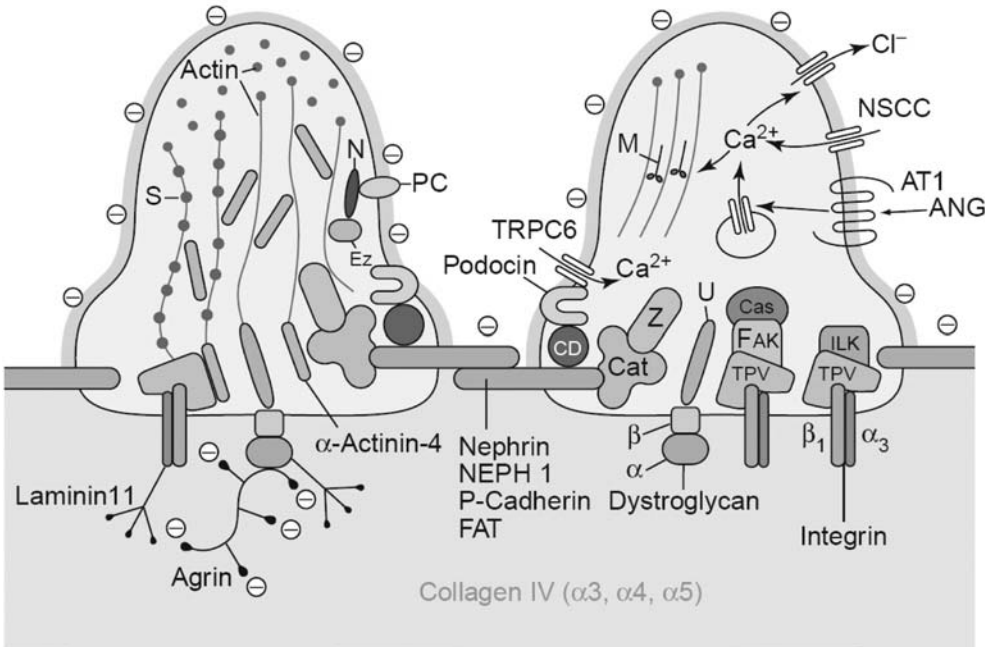
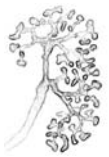


Figure 3. Molecular organization of the podocyte foot process. Two podocyte foot processes with the bridging slit diaphragm are depicted, resting on the glomerular basement membrane (GBM). The central part of the foot process is the actin cytoskeleton (indicated with grey lines and dots), which is reinforced by synaptopodin and α-actinin-4, and has contractile properties as a result of myosin fibers (M). Connected to the cytoskeleton are several molecules that reside in the negatively charged apical membrane, including podocalyxin (PC) via NHERF2 (N) and ezrin (Ez), and podoplanin (not depicted). At the basal membrane, the actin cytoskeleton is connected to the GBM via dystroglycan (linking utrophin (U) to agrin), and the integrin-complex (integrin, talin-paxillin-vinculin (TPV), integrin linked kinase (ILK), focal adhesion kinase (FAK)). The slit diaphragm is composed of nephrin, NEPH-proteins, P-cadherin, and FAT, and the slit diaphragm domain contains several molecules that play a role in the anchoring and signaling of the slit diaphragm (TRPC6, podocin, CD2AP (CD), β-catenin (cat), and ZO-1 (Z)). The podocyte has several receptors, including the angiotensin II receptor AT1. See text for further details. Adapted from (505) and (506), with permission from Elsevier, Lippincott Williams & Wilkins, and the author.



Since the discovery of nephrin several other molecular components of the slit diaphragm have been identified. The nephrin homologues NEPH1-3 were identified in the mouse. As is the case with nephrin, NEPH1 deficient mice show proteinuria and neonatal death (100). Several groups subsequently showed that NEPH1 and nephrin form homo- and heterodimers, suggesting a shared role in formation of the slit diaphragm (101,102). Injection of individually subnephritogenic doses of antibodies against nephrin and NEPH1 induced proteinuria (103), underscoring the functional link between these two molecules. NEPH3 (syn. filtrin, KIRREL2) is expressed at the slit diaphragm region, and its expression was found to be reduced in acquired proteinuric diseases, both at the protein and the mRNA level (104,105).

In a search for other cell-cell adhesion proteins that may be of relevance, Inoue et al found that the protocadherin FAT1 is expressed at the site of the slit diaphragm (106). Mice that lack FAT1 develop, among other abnormalities, proteinuria and foot process effacement (107).

Anchoring to the actin cytoskeleton

The slit diaphragm is inserted in a highly organized membrane region modified by lipid rafts. Podocin is one of the proteins that localizes to this region, and like nephrin is present in lipid rafts (108-110). The protein has a hairpin structure, with both the N- and C-terminus ending in the cytoplasm. Mutations in NPHS2, the gene encoding podocin, lead to a steroid-resistant form of nephrotic syndrome (111). Podocin interacts with several components of the podocyte foot process, including nephrin and CD2-associated protein (CD2AP). TRPC6, coding for a cation channel with a preference for Ca^{2+}, was recently found to be mutated in patients with a late-onset form of FSGS (112). Subsequent analysis showed that TRPC6 localizes to the slit diaphragm region, and associates with other components such as nephrin and podocin (113,114).

As pointed out before, the different membrane compartments of the podocyte are linked to the subcortical actin cytoskeleton. This is also true for the slit diaphragm region. FAT1, for example, was shown to recruit Ena/VASP proteins that play a role in actin polymerization (115). P-cadherin forms a multimolecular complex with α-, β-, and γ-catenin and ZO-1, both ZO-1 and β-catenin could link this complex to the actin cytoskeleton (84). Nephrin interacts with a wide array of intracellular molecules that relate to actin dynamics. One of the first intracellular linkers to be discovered was CD2AP (116), a protein that directly connects to the actin cytoskeleton (117). Studying the role of this protein in the immunological synapse, it was serendipitously discovered that absence of CD2AP in mice leads to heavy proteinuria (118), which brings functional relevance to the nephrin-CD2AP-actin interaction. Other actin-associated molecules that interact with nephrin include IQGAP1, spectrins, and α-actinin-4, while nephrin also connects to MAGUK family proteins that link it to other signaling and cell-junction molecules (119).

Signaling at the slit diaphragm

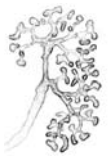
The studies mentioned so far have established numerous structural components of the slit diaphragm and their intracellular connections; a major finding was that several of the proteins located in the slit diaphragm region participate in signaling pathways. Signal transduction in cells is mostly regulated by the attachment or detachment of phosphate groups on serine, threonine, or tyrosine residues of proteins, enabling the binding of other proteins (120). Both the intracellular (C-terminal) part of nephrin and NEPH1 contain tyrosine residues that can be phosphorylated by kinases such as Fyn; the importance of this is underscored by the fact that Fyn knockout mice show foot process effacement and proteinuria (121). Nephrin tyrosine phosphorylation results in activation of AP1, an effect that is enhanced by binding of nephrin to podocin (122). Later experiments showed that in vivo, phosphorylated nephrin in conjunction with CD2AP activates PI3 kinase. This in turn initiates a series of phosphorylations that may lead to intracellular responses including cell survival, actin dynamics, proliferation, metabolism, and endocytosis. Two examples that give some insight in the importance of signaling for podocyte integrity are listed here; more in-depth reviews are provided in references (120) and (123). One specific response is the regulation of apoptosis: nephrin and CD2AP induced PI3 kinase activity may increase AKT expression, thus suppressing TGF-β signaling and preventing podocyte apoptosis (124,125). As will be discussed in a later paragraph, loss of podocytes is linked to the progression of renal disease (126). Secondly, two different groups showed that nephrin phosphorylation by Fyn is crucial for its binding to the adaptor protein nck (127-129). Nck is able to recruit a protein complex that regulates actin polymerization. This suggests that the phosphorylated nephrin – nck interaction may be of importance in states with high dynamic activity such as development and foot process effacement or rearrangement (128-130).

The different interactions between the proteins in the slit diaphragm region, both in physical attachments of proteins and in signaling, make clear that protein complexes rather than single molecules are responsible for a correct podocyte and slit diaphragm function. And not only proteins, but also lipids may be involved. The role of lipid rafts in the cell membrane has already been alluded to; in addition, recent studies by Huber et al indicate that the regulation of TRPC6 by podocin requires binding of cholesterol by podocin (114).

Interaction with the GBM

At the basal site, the 'sole' of the foot process, the podocyte attaches to the matrix they have in part themselves produced: the GBM. This interaction is accomplished by several transmembrane matrix binding proteins, including integrins and dystroglycan.

Integrins are heterodimers, consisting of an α and β subunit, that attach to extracellular matrix molecules. The podocyte's integrin is made up of the α3 and β1 integrin subunit that attach to collagen, fibronectin, and laminins in the basement membrane (131). Blocking the β1 integrin



in animal models leads to proteinuria. α3 integrin knockout mice die in the neonatal period and show defects in lung maturation and kidney development, including a disorganization of the GBM and a failure to form podocyte foot processes (132). Upon extracellular ligand binding, integrins cluster to form so-called focal adhesions. At the intracellular side these focal adhesions recruit several adapter molecules, including talin, paxillin, and vinculin, which attach the integrins to the cortical actin network. As with the slit diaphragm, these interactions serve not only a structural function, but are also involved in signaling pathways. Integrins are able to mediate both inside-out and outside-in signaling, an action in other cells frequently mediated by phosphorylation through focal adhesion kinase (Fak). In podocytes, this route has not been unequivocally demonstrated. Another candidate for mediating the signaling by integrins could be integrin-linked kinase (ILK). Using differential display analysis, the groups of Holthofer and Kretzler found an increase of – among other molecules – ILK mRNA expression in glomeruli of patients with CNF (133). Subsequent experiments showed that ILK mRNA expression was increased in several proteinuric kidney diseases in vivo (134), and that increased ILK activity was related to reduced matrix adhesion in vitro (135). Also, clustering of integrin receptors by ECM molecules leads to a decrease in ILK activity, the outside-in route. Further studies identified several molecules interacting with ILK, linking this complex to actin cytoskeleton dynamics, hypoxia signaling (via HIF-1α and VEGF), the wnt signaling pathway, and proliferation (136-138). ILK activity also induces expression of matrix metalloproteinase 9 (MMP-9) that has a role in GBM remodeling. Podocyte-specific ILK knockout mice showed a normal development, but after three weeks started to become proteinuric. The initial changes were primarily found in the GBM, indicating a possible role for ILK in integrin mediated GBM organization (139). Using a comparable mouse model, another group showed that disruption of ILK signaling also resulted in changes at the slit diaphragm (140).

Apart from integrins, dystroglycans have been implicated in the embedding of the podocyte foot processes in the GBM. Indeed, dystroglycan seems to be well-adapted to such a function: the extracellular domains bind ligands including laminin, agrin, and perlecan – all present in the GBM; the intracellular domain (the transmembrane β subunit) is linked to the actin cytoskeleton via the adapter protein utrophin (50,141). Reports on the localization of dystroglycan in the podocyte have been controversial; some studies reported an expression limited to the basal foot process membrane (142), while others also found dystroglycan expression at the apical membrane (74,141). Raats et al found utrophin expression only at the basal membrane (141), suggesting this to be the place of a dystroglycan-mediated GBM-actin association. Loss of dystroglycan expression at the basal part of the foot processes has been reported in minimal change disease (as opposed to FSGS) that was reversible after steroid treatment (142). A similar finding was reported in a patient with proteinuria but otherwise with no glomerular abnormalities (143). Raats et al instead reported an increase in basal membrane dystroglycan expression in adriamycin nephropathy (141). Later studies have added to the notion that the dystroglycan-GBM connection is of

importance for the correct function of the glomerular filtration barrier: reactive oxygen species were found to decrease the adherence of dystroglycan to agrin, possibly leading to podocyte detachment (75). In a comparable study, Kojima et al found that protamine as well as reactive oxygen species disrupted the link between dystroglycan and its ligands (144). This resulted in a disorganization of the lamina rara externa of the GBM, substantiating the hypothesis, as put forward by Kerjaschki (50,142), that via dystroglycan the actin cytoskeleton of the podocyte may function as a blueprint for the organization and spacing of GBM proteins.

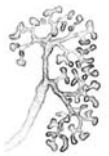
Other proteins that are located at the basal membrane include megalin/gp330 (in rats) and neutral endopeptidase/CD10 (in humans). These proteins have been discovered to be the pathogenic antigens in Heymann nephritis and neonatal membranous nephropathy, respectively (145-148). Megalin may be linked to the cytoskeleton via the adaptor protein MAGI-1 (149).

Podoplanin has also been reported to be present in the basal membrane of the foot processes, its predominant localization being at the apical membrane domain. As discussed above, the intracellular linkers of podoplanin in the podocyte have not been identified, nor is the function of podoplanin at this location clear. In lymphatic endothelial cells podoplanin has been reported to play a role in the shaping of a gradient of the chemokine SLC/CCL21 (150), which is also expressed in podocytes, and which is important for mesangial function (151). It is tempting to speculate that podoplanin is important for the mediation of this cross-talk between podocytes and the mesangium.

Exocytosis of ECM molecules and matrix modifying enzymes such as metalloproteinases takes place at the basal membrane of the podocyte. Also, growth factors such as VEGF are excreted at the basal site of the podocyte, although little is known about the kinetics and precise mechanisms by which this occurs (152). But interactions at the basal membrane of the podocyte are not restricted to binding and modifying the GBM, several other interactions take place. For example, there is extensive endocytosis in this membrane compartment, as can be inferred from the widespread presence of clathrin-coated pits and vesicles (14,153). In vitro, podocytes were shown to endocytose albumin, possibly important in clearing the glomerular filter from macromolecules (154). Others have also found that the podocyte is able to perform transcytosis, yielding a transcellular rather than paracellular, ie, slit diaphragm, route between the intracapillary lumen and Bowman's space (155).

Receptors and signaling pathways

Numerous receptors and coupled signaling pathways that are involved in podocyte function have been investigated, but will not be discussed in detail here. For further details on this subject the reader is referred to an extensive review (14). The bottom-line of the different receptors is the notion that intracellular second messengers, including cyclic AMP, cyclic GMP, and Ca2+ and their related pathways in the podocyte are modifyable by a wide range of extracellular and circulating



molecules. These include vasoactive compounds as nitric oxide, atrial natriuretic peptide, hormones (156), and medication (including dexamethason (157) and cyclosporine (158)). Of some importance, also from a clinical point of view, is the fact that podocytes carry both types of the angiotensin II receptor, AT1 and AT2, suggesting podocytes have a local renin-angiotensin system (RAS) (159). It is well known that ACE inhibitors or AT1 antagonists have a beneficial effect on the kidney, which is not completely explained by their blood pressure lowering properties (152). The inhibition of angiotensin II receptor mediated effects may be the explanation for these observations. Indeed, hypertrophy in podocytes was prevented by ACE inhibition (14), and several other studies have shown a 'podoprotective' effect of RAS inhibition (160-163). Conversely, transgenic rats that overexpress the human AT1 receptor specifically in podocytes develop albuminuria, podocyte foot process effacement, and eventually FSGS (164).

Rastaldi et al found that podocytes express several molecules associated with neuronal synaptic vesicles, and showed that in podocytes these molecules also associate with vesicles (165). These findings expand the extent of similarities between neurons and podocytes. Indeed, the branched appearance, cell cycle quiescent phenotype, cytoskeletal organization (166), and gene expression pattern (167) of these cells are strikingly similar. Moreover, these findings could indicate that apart from intracellular signaling, also an intercellular communication by means of synaptic-like exocytosis of glutamate may take place in podocytes (168).

Cell cycling and transcription factors

During the capillary loop stage of glomerular development, podocytes start to differentiate, they form foot processes and express typical podocyte markers. At the same time these cells stop to proliferate. In the mature glomerulus, podocytes are considered to be terminally differentiated, post-mitotic cells. Proliferation, or progression through the cell cycle, is regulated by a complex set of stimulatory and inhibitory proteins. Cyclins and their respective cyclin dependent kinases (CDKs) promote proliferation, while CDK inhibitors such as p21, p27, and p57 inhibit proliferation. In podocytes, an upregulation of CDK inhibitors is seen in the capillary loop stage, promoting a quiescent podocyte phenotype. Also in response to injury, podocytes, in contrast to other glomerular cells such as mesangial cells, do not divide, although they do show hypertrophy and sometimes multi-nucleation. Petermann et al found that in response to injury, podocytes do enter the cell cycle: they show (limited) DNA amplification and upregulate proteins that mark the start of the cell cycle. However, there was no proliferation of podocytes, suggesting that they do not have the ability to complete the cell cycle and perform cytokinesis (169). Others have suggested that the complex cellular architecture of podocytes prohibits cytokinesis (14). Consequently, there must be cell cycle inhibitory molecules that prevent cell division. Loss of such inhibitory regulators, as exemplified by the p21 and p27 knockout mice, results in podocyte proliferation in response to damage (170,171). While loss of podocytes is considered to be the initial step towards

nephron loss, also certain glomerular diseases have been associated with a proliferating podocyte phenotype. Almost all of these diseases show a detrimental course when left untreated. Moeller et al studied transgenic mice with tagged podocytes and showed that podocytes contribute to cellular crescents, seen in some forms of glomerulonephritis (172). In HIV-associated nephropathy podocytes are presumed to be able to escape their cell-cycle control and re-proliferate, leading to FSGS of the collapsing type with a rapidly progressive clinical course (173-175). It has been difficult to prove that these cells are really podocytes – the cells are dedifferentiated, they have lost their typical markers and they are allegedly podocytes merely on the basis of their localization in the glomerulus. Studies by Dijkman and Smeets et al brought evidence for the role of parietal epithelial cells in proliferative glomerular diseases (34,39).

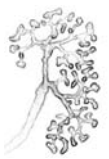
Transcription factors that regulate podocyte development and maintenance include PAX2, pod1, Kreisler, Lmx1b, and WT1 (176). WT1 has been linked to the expression of several podocyte markers, including nephrin and podocalyxin (177-180). The crucial role for WT1 is underscored by the fact that mutations in the gene cause syndromes (Denys-Drash, Frasier, and WAGR syndrome, OMIM 607102) that frequently involve podocyte and glomerular abnormalities.

Mutations in the gene that encodes the transcription factor Lmx1b cause nail-patella syndrome, characterized by the absence of the patella and nails and by the occurrence of nephropathy. Two groups of investigators studied Lmx1b knockout mice and found that this transcription factor is important for the expression of collagen α3(IV) and α4(IV), and the slit diaphragm associated proteins podocin and CD2AP (181,182), indicating a role for this transcription factor in both GBM formation and slit diaphragm function.

Glomerular filtration: characteristics and theoretical models

How exactly the kidneys produce urine has been investigated for over 150 years and remains unresolved. Initially, the question was whether the glomerulus takes at all part in formation of urine. At that time, tubular secretion probably was a more plausible option, as secretive epithelia in salivary, gastrointestinal, lactating glands etc. had just been extensively studied. The notion that urine is formed by glomerular filtration and tubular resorption and excretion was only proven through micropuncture studies in the 1920-60s (183-185). Still, the molecular mechanism of glomerular filtration remains incompletely understood.

In this paragraph, the characteristics of the glomerular filtration barrier are described: the amount and concentration of fluids, small solutes, and macromolecules involved, the forces that drive filtration, and the biochemical and biophysical properties of the filter. This is followed by a description of several theories on how glomerular permselectivity is accomplished at subcellular and molecular levels.



Characteristics of glomerular filtration

Amounts and concentration of fluids

The kidney receives about 20 percent of the cardiac output, ~1.2 liters of blood per minute. Some ten percent of this total volume is filtered in the glomerulus, and enters the tubular system as pre-urine; the total volume of pre-urine thus amounts to 180 liters per day. Most of the pre-urine is reabsorbed in the tubules, leaving an average of 1.5 liters of urine each day for excretion (range 0.8 – 20 liters). The ultrafiltrate has almost the same composition as plasma-water, it is acellular and contains a low amount of protein. It is generally agreed upon that the concentration of albumin in the pre-urine is about 25 µg/ml, compared to a plasma albumin concentration of 45mg/ml. This indicates that the glomerular filter is restrictive for proteins, a feature referred to as glomerular permselectivity. The extent of restriction differs for each protein (see below), and is expressed as the glomerular sieving coefficient (Bowman's space-to-plasma concentration ratio) θ, theta.

Forces that drive filtration

There are different forces that drive transport of fluids through the glomerular capillary wall. These so-called Starling forces include the hydrostatic and colloid osmotic pressure, determined by the fluid-pressures and the colloid osmotic value of the fluids, respectively. These two forces work in opposite directions: the hydrostatic pressure in the glomerular capillaries is higher than that in Bowman's space, providing an outward force. Instead, the colloid osmotic pressure within the capillaries exceeds that in Bowman's space, and this will drive transport of fluids inwards. In the upstream part of the glomerular capillaries, the hydrostatic outward force is higher than the colloid osmotic inward force, resulting in filtration. Since the filtration barrier is restrictive to proteins, the extraction of fluids from inside the capillaries will increase the intracapillary protein concentration, and thus the colloid osmotic pressures. Eventually, in the downstream part of the glomerular capillaries this will lead to an osmotic inward force that equals the hydrostatic outward force, the so-called filtration pressure equilibrium. Downstream of the point where the filtration pressure equilibrium is reached, there is no filtration.

The amount of filtration is further influenced by the characteristics of the filter, represented in the ultrafiltration coefficient K_f, the constant that indicates the resistance to fluid flow through a barrier. Higher levels of K_f indicate a higher permeability. Both the GBM and the cellular constituents of the filter contribute to the resistance to flow.

Small molecules are mainly transported by convection, and thus hold pace with the transport of the fluids over the capillary wall, while macromolecular transport is determined by both convection and diffusion.

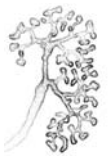
Biochemical and biophysical properties of the glomerular filter

The general view is that the glomerular filter restricts passage of macromolecules on the basis of their size, shape, and charge. The influence of these factors determines the sieving coefficient θ

for each macromolecule. Because macromolecular transport is passive, θ varies between 1 (free passage through the filtration barrier) and 0 (complete restriction). The different determinants of glomerular permeability will be discussed here.

Size selectivity – The size of a molecule, commonly indicated by its Stokes-Einstein radius (SE radius), is influenced by the compactness of the molecule and its molecular weight. For albumin (molecular weight 3000 kDa, SE radius 36 Å) the sieving coefficient θ is about $6 \cdot 10^{-4}$ (25 µg/ml, the albumin concentration in Bowmans' space divided by 45 mg/ml, the plasma albumin concentration). The θ for smaller proteins is considerably larger, and proteins smaller than 14 Å have a θ that is 1, indicating that these molecules are not restricted by the glomerular filtration barrier. For larger proteins such as IgG (molecular radius 55 Å) or IgM (molecular radius 120 Å), the θ is considerably smaller; these molecules may even be completely absent from the ultrafiltrate in Bowman's space. The exact size selectivity of the glomerulus has been difficult to determine: direct measurement of proteins should be performed in the glomerular ultrafiltrate, before reabsorption in tubules may occur. This requires micropuncture techniques that have been criticized because they may measure proteins released as a result of the tubular damage associated with the measurement itself, or by contamination of peritubular capillary blood proteins. The most reliable direct measurements of protein concentrations are probably experiments by Tojo and Endou (186), who used sophisticated techniques to circumvent the problems associated with micropuncture techniques. These studies have established the before mentioned θ for albumin of $6 \cdot 10^{-4}$. Furthermore, patients with Fanconi syndrome have been used for estimations of the glomerular filtration selectivity. These patients have an impaired tubular protein uptake, and the protein concentration in urine is thus a reflection of their glomerular protein filtration. In these studies, the θ for albumin was found to be ~$8 \cdot 10^{-5}$, which is even lower than that of rodents (187). Probes of different sizes, such as the polysaccharides dextran and Ficoll as well as different proteins, have been used to get insight in the exact size characteristics of the filter. Results from these experiments have been used in models of glomerular size selectivity, in which the glomerular filtration barrier is considered to be perforated by pores with a certain diameter. In most models, there are two 'pore-populations': a large number of restrictive small pores with a radius between 37 and 55 Å, and far less frequent unrestrictive large pores or 'shunts', with a radius of 80 – 100 Å (43,188-191).

Macromolecular shape – Uncharged probes with a similar SE radius may show a divergent filtration behavior. For example, the polysaccharide dextran has a θ that is about 7-fold higher than that of an uncharged protein of the same size, and is also larger than Ficoll with the same radius. This is attributed to the fact that dextran may change its conformation from a sphere to a more elongated molecule, and thus pass through the capillary wall more easily. Also for Ficoll, although more spherical than dextran, the glomerular filtration barrier was found to be to some extent



hyperpermeable, probably due to the compressibility of Ficoll (188). This indicates that the form, globular or linear, and deformability of a molecule is of importance for its sieving characteristics.

Charge selectivity – Several studies have demonstrated that the capillary wall restriction for anionic proteins is higher than that for neutral or cationic proteins, indicating the presence of charge selectivity (192-194). The extent of this charge selectivity has, however, been difficult to quantify. This relates in part to the use of tracers that may not truly mimic the behavior of proteins in the capillary wall: negatively charged sulfated dextrans may more rapidly interact with the components of the glomerular filter, thus retarding their passage (188). As pointed out before, the capillary wall seems to be hyperpermeable for Ficoll in comparison to albumin of the same size. This has led to the suggestion that the negative charge of albumin accounts for the apparent restriction, which would fit with an important charge barrier (195), although the divergent behavior may in fact be related to the compressibility of Ficoll. Furthermore, comparison of tracers such as sulfated dextrans and carboxymethyl Ficoll (tracers that have been rendered negatively charged) with their neutral counterparts even showed an increased permeability of negatively charged tracers (196). Removal of molecular constituents of the negative charge in the GBM – using enzymatic and genetic methods – failed to induce proteinuria, further questioning the role of the GBM in charge selectivity (197-201). It has been suggested that the actual charge barrier may reside in the endothelium rather than in the GBM (see below). Also, a charge effect could be built up by negatively charged components of the blood, such as albumin, which accumulate during filtration or interact with the endothelial cell glycocalyx (202). An indirect proof for this is the observation by Ryan and Karnovsky, that a continuous blood flow is needed to maintain a normal filtration barrier (203). Direct proof for this mechanism is lacking. Thus, although most authors acknowledge the presence of charge selectivity, at least with regard to the filtration of proteins, its relative contribution to permselectivity remains controversial.

Different explanations for permselectivity

The description of the functional characteristics of glomerular filtration directly relates to the question how filtration is accomplished on a structural level. In the literature, the different structural components of the glomerular filter have all been given attention, with a recent skewing towards the contribution of the podocyte. An overview of the different explanations and hypothesis is lined out below.

The GBM as the main filter for plasma proteins

In 1975, Farquhar in a review on glomerular filtration concluded that 'the bulk of the evidence available at present favors the basement membrane as the primary filtration barrier in the glomerulus' (204). Indeed, in most tracer studies, an accumulation of tracer molecules at the subendothelial rather than at the subepithelial side has been observed. On the molecular level, the GBM contains proteins that permit the formation of sieve-like structures, for example through

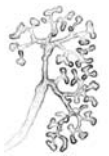
cross-linked collagen IV networks, and removal of some essential components leads to the development of proteinuria. Localization of the charge restriction in the GBM has been inferred from the observation of regularly spaced anionic sites demonstrated using for example polyethyleneimine, and the corresponding molecules have been identified as the glycosaminoglycan sidechains of proteoglycans such as agrin, perlecan, and collagen XVIII. In conclusion, charge and size selectivity can both be explained by the structural and molecular properties of the GBM. Yet, this view leaves several observations unexplained. For example, while IgG is mostly absent from the glomerular ultrafiltrate, injection of IgG directed against nephrin and megalin binds these antigens expressed on the podocyte membrane (85,145), indicating that these IgGs have passed through the GBM. The contribution of GBM proteoglycans to the charge selectivity has been criticized by investigators pointing out that in other tissues such as cartilaginous tumors albumin is found in the stroma, which is even more rich in proteoglycans than the GBM (205,206).

Furthermore, fitting data obtained from experiments with isolated GBM into a theoretical model of glomerular filtration, Deen et al concluded that the contribution of the GBM to permselectivity is relatively small in comparison to that of the cellular components of the filter (26,207,208). These could then either be the endothelial or the epithelial cells. Over the last decade, the contribution of the podocyte to the permselectivity of the glomerular filter has received most attention.

The podocyte slit diaphragm is the main barrier for plasma proteins

Tryggvason and coworkers, after the discovery of nephrin, argued that the podocytes and especially the slit diaphragm would be the main site of ultrafiltration (95,209-211). This hypothesis was supported by observations by Rodewald en Karnovsky in the 1970's, who observed that the slit diaphragm had a zipper-like ultrastructure and suggested that this could explain the glomerular size selectivity (83). Using electron tomography, Wartiovaara et al visualized nephrin strands spanning the slit pore and leaving lateral pores of about the size of an albumin molecule (96). The fact that absence or abnormal function of many other slit diaphragm-associated proteins leads to proteinuria brings additional evidence for the importance of the slit diaphragm in glomerular permselectivity, and possibly for its dominant role in ultrafiltration.

If, however, the most selective barrier is indeed localized downstream in the filter, one would expect that proteins that pass through the upstream layers would 'pile up' in the sub-epithelial part of the GBM, a phenomenon called concentration polarization (202). In other words, the filter would clog (152,202,211,212). Several mechanisms that prevent such a clogging of the filter have been put forward. For example, the characteristics of the previously mentioned subpodocyte space theoretically leave open the possibility of an inversion of the direction of the fluid flow in the capillary wall, and could thus play a role in the unclogging of the filter (29,30). Another recently pursued hypothesis is that the proteins that accumulate at the basal membrane of the podocyte are transported to Bowman's space by transcytosis (155). Indeed, coated pits



are frequently observed at the basal membrane, and in vitro podocytes have been shown to be capable of large scale endocytosis (154).

Alternatively, the restrictive properties of the glomerulus could be localized to the other cellular component, the endothelium.

The endothelial cells restrict passage of proteins

Deen showed that theoretically the endothelial layer is able to contribute notably to the glomerular filtration barrier (208). Early studies of glomerular permselectivity had ruled out a contribution of the glomerular endothelium, as it was recognized that the fenestrations would be too large to restrict passage of macromolecules (204). Later studies, however, have changed this view. Rostgaard and Qvortrup used special fixation and staining techniques that allowed them to study the ultrastructural organization of the glomerular capillary wall in the absence of changes that would be caused by lower blood pressure or anoxia. They found that the endothelial cells are covered with a 300nm thick glycocalyx, and that the fenestrae of the endothelial cells were bridged by filaments (25). In a later study, the same authors described the presence of a surface coat, presumably made up of proteoglycans on the endothelial cells, which extended into and filled up the endothelial fenestrations (213). This led them to hypothesize that these 'sieve plugs' or 'fascinae fenestrae' would be the actual basis for the glomerular permselectivity (213). Others have shown that the glomerular endothelial cells produce negatively charged proteoglycans (214). Taken together, the glomerular endothelial cells may indeed play a more important role in glomerular filtration than has been generally acknowledged (43,202,208,211).

Integrative views of glomerular filtration

Some explanations bring a more integrative view of the filter. These include the view of the glomerular filration barrier as size and charge barriers in series, the 'Electrokinetic glomerulus theory' by Douglas Somers, and Oliver Smithies' 'Permeation diffusion hypothesis'.

Size and charge barriers in series

In the classic view of the glomerular filtration barrier, the GBM and the podocyte slit diaphragm are two size and charge selective barriers that are placed in series. The GBM functions as a coarse filter for the larger molecules, while the slit diaphragm is the fine filter (204,215). If this view of the glomerular filtration barrier were correct, this would lead to a concentration polarization, ie, a clogging of the filter. Thus, this view of glomerular filtration seems to suffer from the combined inconsistencies mentioned in the discussion of the individual components of the filter.

Electrokinetic glomerulus theory

In Douglas Somers' 'Electrokinetic glomerulus theory' (Somers D, J Am Soc Nephrol 2005 (16): 109A), the central tenet is that anion transport over the GBM occurs more easily than cation transport, because the latter will continuously interact with the fixed negative charges of basement membrane components. This transport imbalance will result in the accumulation of

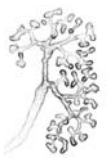
negative charge at the urinary space side of the GBM. In steady-state conditions, this will lead to a charge gradient with the highest negative charge downstream in the GBM. The increasing negative charge provides the restriction to negatively charged molecules such as albumin and, according to this model, explains the glomerular filtration characteristics. The observation that the concentration of molecular tracers was higher in the subendothelial than the subepithelial part of the GBM (193,216) has been explained to support this view of an increasingly restrictive barrier, although other explanations are possible for this finding.

Because ion transport takes place by convection, the formation of the charge gradient is dependent on flow. Flow, as described above, is dependent on the filtration pressure characteristics in the glomerulus: the combined pressure and osmotic forces that drive the fluids out of the capillaries minus those that would drive fluids in. Halfway the glomerulus the in- and outward forces are similar ("raising the question why we have an efferent capillary in the first place"), resulting in a filtration pressure equilibrium. Consequently, in the glomerular capillaries downstream of the filtration pressure equilibrium, there will be no flow and thus no negative charge will build up. This would result in a loss of albumin by simple diffusion through the GBM. To explain the fact that this does not seem to occur, Somers suggests that the podocyte, by crossing the space between different capillaries, may transport the negative charge built up in afferent capillaries to efferent capillaries. This is compatible with the negatively charged surface of the podocyte's major and foot processes that would prevent a loss of charge through an 'electric shortcut'.

One of the predictions of this model is that proteinuria will occur in situations where GFR is decreased, because the subepithelial negative charge will not build up completely. Indeed, Rippe et al recently found that the sieving coefficient of Ficoll is increased (meaning that Ficoll is less effectively retained by the glomerular barrier) in low GFR conditions (217).

Permeation/diffusion hypothesis

In the Permeation/diffusion hypothesis (212), the size-selective properties of the glomerular filtration barrier are thought to reside in the GBM. Smithies assumes that the GBM can be viewed as a gel, and argues that – as in all gels – only a limited fraction of gel space is available for macromolecules, determined by factors such as concentration of the gel and radius of the macromolecules. The small amounts of macromolecules that can permeate into the gel are subsequently transported across the GBM by either flow or by diffusion. With regard to the GBM-gel, the fraction available for albumin is about 0.02. Smithies calculates that the main means of macromolecular transport in the glomerulus is diffusion. In contrast, transport of water and small solutes depends on flow. Changes in flow have no effect on the diffusion, and will thus have only a minimal effect on the amount of albumin in Bowman's space. However, the concentration of albumin is greatly influenced by flow. A decrease in glomerular flow (GFR) will lead to an increase in albumin concentration, saturating the tubular albumin reabsorption mechanisms and thus leading to



albuminuria. In other words, the transport of albumin into Bowman's space is fairly constant, but the absence of the diluent is responsible for proteinuria. In this view, albuminuria is not necessarily the result of a pathologic change in the glomerular filtration barrier, but may be the physiologic result of a decrease in GFR. On the other hand, changes in the glomerulus do induce albuminuria if they increase the hydrodynamic resistance of the filtration barrier, as for example in podocyte foot process effacement (218), or if they change the properties of the GBM (219). The experiments by Rippe et al, showing increased sieving coefficients for Ficoll in low-GFR conditions, are in line with this theory of glomerular filtration.

In general, rodents have a relatively higher urinary protein excretion than humans. This could possibly be explained to be in line with the permeation diffusion theory: if the protein concentration is determined by the diffusion of albumin and the filtration of the diluent, the factors that determine these two variables are, respectively, the surface available for diffusion, and the amount of filtrated fluids. Human glomeruli are larger than those of rodents, and thus the diffusion surface and fluid content of human glomeruli is also larger than that of rodents. But diffusion surface increases with a factor to the power 2, while fluid content increases with a factor to the power 3. Thus more diluent is available, suggesting that larger glomeruli would elaborate a more dilute pre-urine.

The permeation-diffusion theory does not readily explain the proteinuria seen in situations with a normal GFR and normal foot process morphology, as has been observed in animal models and sporadic human cases (85,103,143,220).

Absence of glomerular permselectivity

The most extreme view of glomerular filtration is that there is no, or at least very limited glomerular permselectivity, a hypothesis that has been fueled by several studies of Comper and coworkers. Their hypothesis explains the inconsistencies that remain in the other theories concerning glomerular selectivity, but brings up another problem: if the glomerulus would filter 'nephrotic levels of albumin' (221), this would result in an albumin excretion of about 600 g per day (202). To explain the fact that only limited amounts of this reach the urine, Comper et al have hypothesized that there is a high capacity albumin retrieval pathway in the proximal tubulus. Over the years, Comper et al have brought evidence for the absence of glomerular size and charge selectivity (205), the presence of tubular retrieval pathways for example through tubular transcytosis (221), and have raised a hypothesis to explain proteinuria seen in glomerular diseases (222). Although the potential interest of their findings is acknowledged (223), the methods of the experiments have been criticized (14,43,202,223,224) and their results could not be reproduced (190).

Synthesis

Most reviews on the subject of permselectivity move towards the recognition that the three layers of the glomerular filtration barrier are functionally interdependent, making it difficult to attribute

a more important role to one of the three (225). Indeed, damage to any of the three layers can be associated with the development of proteinuria (see below). Although intuitively true – why else would the glomerular capillary wall be endowed with its special properties – this view leaves the question as to how exactly the glomerulus elaborates its low-protein ultrafiltrate unanswered.

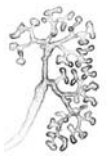
From Bowman's space, the glomerular ultrafiltrate empties into the tubular lumen, where its composition is modified on its way to the collecting ducts. The tubular system will be discussed next.

The tubular system

Each glomerulus is connected to a ~5 cm long renal tubule that is built of a proximal, intermediate, and distal segment. The proximal segment starts as a contortuous tube, containing cells with a prominent brushborder, and connects to a straight part. This part of the proximal tubule forms the transition to the intermediate segment that loops through the medulla as Henle's loop. Ascending from the medulla, Henle's loop connects to the distal convoluted tubule that on its path to the collecting duct makes contact with the glomerular arterioles at the macula densa (226).

The different segments of the tubule contain cells with a different morphology and accordingly different functions. The main function of proximal tubular cells is reabsorption of water and the dissolved salts and small molecules of the ultrafiltrate. To this end, the cells are equipped with microvilli that provide a ~40 times increase in apical surface area. At the beginning of Henle's loop, the amount of ultrafiltrate has been reduced to about 70 percent. The cells in Henle's loop further reabsorb water and salts, with a predominance of salts, thereby increasing the medullary salt concentration. This forms the basis for the concentration of urine that is eventually performed in the collecting ducts. The distal collecting duct further reabsorbs NaCl as well as calcium. Water is less efficiently reabsorbed in this segment, resulting in a hypoosmotic fluid that is delivered into the collecting duct.

The reabsorption and excretion mechanisms are extensively regulated throughout the course of the tubules by hormonal factors. Of further interest for the regulation is the juxtaglomerular apparatus, in which the macula densa of the distal segment takes part. This apparatus is – via incompletely elucidated mechanisms – responsible for the so-called tubulo-glomerular feedback that regulates the glomerular filtration in relation to the distal tubular output. The exact transport and regulation mechanisms at play in the tubular system will not be further dealt with in this



introductory chapter. In the interest of a discussion on the effects of filtered proteins on tubular epithelial cells, the mechanisms of tubular protein resorption will be discussed in some more detail.

Proteins are mainly reabsorbed in the proximal tubules through receptor-mediated endocytosis. The main receptors for albumin in the proximal tubule are cubulin and megalin. Both are multiligand endocytic receptors that are able to bind albumin. Binding of albumin to these receptors is followed by endocytosis and transport of the endocytic vesicles to the lysosome. Here albumin is degraded into amino acids that are subsequently excreted in the PTCs, while the receptors are recycled to the apical membrane (227,228). One research group has found that other ways of albumin transport may exist, namely through transcytosis (221). The endocytosed albumin is transported through the cell and delivered undegraded into the circulation. This way of albumin transport awaits further confirmation.

The tubular epithelial cells manufacture and rest on a basement membrane (TBM) of collagen IV (isoforms $\alpha1\alpha1\alpha2$), laminins (111 and 511), and HSPGs (mainly perlecan).

Interstitium and the extracellular matrix

Between tubuli and glomeruli is the interstitium, which contains the blood vessels, peritubular capillaries (PTCs) as well as lymphatics. These vascular structures are embedded in a loose extracellular matrix, produced by the interstitial fibroblasts. Other interstitial cell types include macrophages and – more recently described – dendritic cells that form a network surrounding the tubuli and glomeruli (229,230).

The normal interstitial matrix contains collagens type I, III, V, VII, fibronectin, and tenascin. These molecules are present only in small amounts, for in the normal kidney cortex the interstitial space is limited: tubuli are normally positioned side by side, and the intervening PTCs fill most of the remaining space, often fusing their basement membranes with the TBM, at least optically.

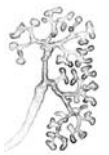
Lymphatics accompany the larger arteries of the kidney, but are otherwise not found in the normal interstitium.

PART 2 | CAUSES AND CONSEQUENCES OF PROTEINURIA

Proteinuria, the presence of abnormally high amounts of plasma proteins in the urine, is a symptom associated with many different kidney diseases. Although the causes of proteinuria are diverse, the consequence of proteinuria in different diseases is similar, in that it confers an increased risk of loss of kidney function, in association with an increased risk of cardiovascular complications. This, together with the fact that the development of proteinuria may be modifiable, makes it an important risk factor in both native and transplant renal disease. In this paragraph, a brief overview of the epidemiology of proteinuria and the kidney diseases in which proteinuria is seen is provided. The paragraph focuses on the causes and consequences of proteinuria on the histopathological and molecular levels. The paragraph ends with a more general discussion of the mechanisms implicated in the progression of renal disease.

Epidemiology

Several large population screenings have established the epidemiological characteristics of proteinuria. In the district of Okinawa, Japan, more than 100,000 individuals were screened for proteinuria and follow-up was recorded for more than 20 years (231). From these studies it became clear that proteinuria is an important independent predictor of the development of end stage renal disease. In Groningen, The Netherlands, a large population study, the PREVEND study, was performed in which about 40,000 persons were screened for albuminuria (232,233) (www.prevend.org). These studies made clear that in 6.6 percent of the otherwise healthy population microalbuminuria was present. Furthermore, not only was microalbuminuria found to be associated with the progression of renal disease, it also was an important predictor of cardiovascular diseases (232,233). Several other epidemiologic studies have found a strong correlation between proteinuria and the progression of renal disease (234-236). Based on this association, in 2006 the Dutch Kidney Foundation started the 'Niercheck', a national campaign to detect kidney damage in an early, preclinical stage on the basis of macroalbuminuria measurements (www.nierstichting.nl).



Causes of proteinuria

Proteinuria can either be caused by a failure of tubular reabsorption of the small amounts of filtered proteins, or by a loss of glomerular permselectivity, leading to larger amount of proteins in the pre-urine that saturate the tubular retrieval systems.

Examples of the first include the Fanconi syndrome, in which there is a dysfunctional proximal tubular reabsorption mechanism (187). Such 'tubular proteinuria' usually results in only mild increases in the protein content of the urine. Instead, glomerular proteinuria can be associated with large increases of albumin and other proteins in the urine, and may lead to the development of a nephrotic syndrome (see below).

Glomerular proteinuria can be associated with damage to all different levels of the glomerular filtration barrier, the endothelium, the GBM, and the podocyte.

For example, preecclampsia is associated with endothelial cell damage and profound proteinuria. In the glomerulus, swelling of endothelial cells (endotheliosis) is seen. Pathogenetically, the development of preecclampsia has been linked to the excess production of a soluble VEGF receptor (soluble Fms-like tyrosine kinase, sFlt), that binds and inactivates VEGF. In the glomerulus, VEGF-A produced by podocytes may therefore not reach the endothelial compartment in sufficient amounts, leading to a loss of endothelial viability (21,237,238). Enzymatic breakdown of the endothelial glycocalyx resulted in an increased permeability for proteins that was mainly due to a loss of charge selectivity (239).

The correct molecular make-up of the GBM is crucial for a normal filtration barrier; impaired assembly of the GBM components leads to proteinuria or an otherwise more permeable GBM. This is most clearly underscored by hereditary syndromes that have been associated with mutations in genes coding for GBM components. Patients with Alports syndrome carry a mutation in one of the collagen chains of the mature GBM. Alports syndrome is characterized by hematuria, proteinuria, and loss of renal function (240). Similarly, in nail-patella syndrome, a malfunction of the transcription factor Lmx1b leads to an aberrant collagen IV assemblage, followed by nephrotic syndrome (241). Also, incorrect assembly of the GBM matrix components as seen in the ILK knockout mice (139), is related to the development of proteinuria.

A laminin β2 chain knockout mouse was shown to develop severe proteinuria, despite normal glomerular development and continued expression of the fetal laminin β1 chain (242). The laminin 521 composition thus appears crucial for the correct function of the GBM. In retrospect, the laminin β2 knockout mouse appeared to accurately mimic the characteristics of Pierson's syndrome, a hereditary nephrotic syndrome that was described by Pierson et al in 1963 (243).

Recently, it was recognized that the cause of Pierson's syndrome is a mutation in the gene coding for laminin β2 (244,245).

Strikingly, mice that in a podocyte-specific fashion lack expression of perlecan (200) or large parts of agrin (197,199) – the major heparin sulphate proteoglycans of the GBM – show no proteinuria, although perlecan null mice seem to be more susceptible to glomerular damage (246). Likewise, mice that lack Ext, the protein responsible for heparan sulphate biosynthesis, in podocytes are not proteinuric (201). Also, in vivo degradation of heparan sulphates in the GBM does not cause proteinuria (198). As discussed before, this indicates that the charge barrier of the GBM may be of less importance for the correct function of glomerular permselectivity than previously thought.

Damage of podocytes is almost always accompanied by proteinuria. There are several ways in which podocytes can be damaged, including through genetic, inflammatory, and infectious diseases, through growth factors, cytokines, medication, and mechanical stress. Several of the hereditary forms of proteinuria have already been discussed, as they have been closely related to the elucidation of the cell biology of the podocyte. The currently known forms of hereditary proteinuria are listed in table 1. Other forms of podocyte damage will be illustrated in the description of proteinuria that occurs in various kidney diseases.

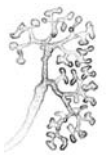
Causes of proteinuria in various kidney diseases

Proteinuria is a symptom of many kidney diseases and is especially seen in diseases characterized by a glomerular involvement. These comprise among others minimal change disease, focal segmental glomerulosclerosis, diabetic nephropathy, membranous nephropathy, membranoproliferative nephropathy, and lupus nephritis. In these diseases, proteinuria can occur as a component of the nephrotic syndrome, characterized by a loss of more than 3,5 gram of protein per 24 hours (nephrotic range proteinuria), edema, and hypoalbuminemia.

How does the development of proteinuria in these different diseases relate to the different processes discussed above?

Minimal change disease

Minimal changes disease (MCD), also termed minimal lesion or lipoid nephrosis, is the main cause of proteinuria in children, and the third most common cause of nephrotic syndrome in adults (247). The presentation is that of a nephrotic syndrome, not infrequently following infections or vaccinations. The condition is generally responsive to treatments with steroids, although



patients may show relapses, and some may even be steroid resistant. In such cases FSGS should be suspected.

The histopathology of MCD, as the name implies, does not show gross abnormalities on light microscopy. On electron microscopic evaluation, extensive effacement of podocyte foot processes is seen. The pathogenesis of MCD remains unknown, although several mechanisms have been hypothesized (247). A T-cell derived permeability factor has been suggested to induce the development of MCD (248), although definitive proof for this is lacking. Several cytokines have been shown to be upregulated in MCD, and these could act via cytokine receptors that have been found to be expressed by podocytes (249). Van den Berg et al (250) found that through the action of IL-4 and IL-13 on podocytes, the activity of the proteases cathepsin L and heparanase in the GBM is increased. This may subsequently lead to a degradation of heparan sulphate in the GBM, leading to proteinuria. Indeed, alterations of the heparan sulphate moieties in the GBM have been repeatedly found in MCD (251).

The group of Bakker and coworkers has postulated that hemopexin might be the circulating factor that is responsible for the development of MCD. Injection of hemopexin in rats resulted in a disorganization of the anionic sites at the subendothelial side of the GBM, and transient proteinuria (252).

Table 1. Hereditary forms of proteinuria

Gene	Disease	Protein	Glomerular morphology	References
NPHS1	Congenital Nephrotic syndrome of the Finnish type	Nephrin	FSGS	(88)
NPHS2	Steroid resistant nephrotic syndrome	Podocin	FSGS	(111)
ACTN4	FSGS1	α-actinin-4	FSGS	(56)
TRPC6	FSGS2	TRPC6	FSGS	(112,113)
CD2AP	FSGS3	CD2-associated protein	FSGS	(502)
PLCE1	Nephrotic syndrome	Phospholipase Cε1	DMS	(503)
LMX1B	Nail-patella syndrome	Lmx1b	DMS, FSGS	(181,182,241)
WT1	Isolated DMS, Denys Drash syndrome, Frasier syndrome, WAGR	Wilms tumor protein 1	DMS/FSGS	(504)
LAMB2	Pierson's syndrome	Laminin β2	DMS	(245)

FSGS – focal segmental glomerulosclerosis; DMS – diffuse mesangial sclerosis; WAGR – Wilms' tumor, aniridia, genitourinary malformations, mental retardation.

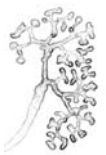
Others have attributed a more direct role to the podocyte in the development of MCD. Patrakka et al found that the slit diaphragm is often lacking in patients with MCD (253). Regele et al (142) found that the expression of dystroglycan was decreased in minimal change disease in comparison to FSGS. Recognizing the role of podocyte-associated proteins in hereditary proteinuric syndromes, several investigators have studied the expression of these proteins in MCD (254-257). The results varied, but seem to be most compatible with a secondary change rather than primary involvement in the development of proteinuria in MCD (258,259). In a scenario in which the podocyte is the key player in the development of MCD, the fact that this disease is responsive to steroids could be explained by the steroid responsiveness of podocytes (157).

Focal segmental glomerulosclerosis (FSGS).

FSGS is also characterized by nephrotic range proteinuria, often in the setting of a nephrotic syndrome. In contrast to MCD, the proteinuria is mostly refractive to steroid treatment, and comes with a progressive decline in renal function that eventually may necessitate transplantation. The histopathology of FSGS is characterized by scarring of some of the glomeruli (focal), in a way that involves only limited parts of a glomerulus (segmental). The lesions that are seen in FSGS are diverse, and recently a classification has been made to describe the different forms of FSGS (260). In this classification, five patterns of FSGS are distinguished on the basis of their morphologic features, namely the collapsing, tip lesion, cellular, perihilar, and not otherwise specified variants of FSGS. Diagnosis of one of the variants requires exclusion of the previous variants in the order listed here. These different variants of FSGS may coexist in a single renal specimen (38), and it is unclear whether the classification on morphologic basis relates to different pathophysiologic mechanisms. The first studies that evaluate the clinical implications of the classification do show differences between the variants; for example, the collapsing variant is associated with a population that differs in demographics from that of the other variants, and clearly carries a worse prognosis (261).

FSGS should not be regarded as a disease in itself, but rather as the stereotypic histomorphological representation of different specific diseases. These include hereditary diseases, obesity, hypertension, viral infection, medication, and mechanical stress. A substantial percentage of cases of FSGS, however, are of unknown origin, the so-called primary or idiopathic forms of FSGS.

In diseases that lead to FSGS, the secondary forms of FSGS and hereditary syndromes, podocyte injury has been shown to be a central step in development of FSGS and the associated proteinuria (262). In hereditary cases, the mutated genes often code for proteins with a more or less specific expression in podocytes, as discussed before. In animal models, direct damage to podocytes, for example through the injection of puromycin aminonucleoside, leads to proteinuria and subsequently development of FSGS. Medication like cyclosporine and pamidronate may damage the



podocyte; the latter gives rise to a collapsing variant of FSGS with high proteinuria (263). Podocytes have been shown to be a target of infection by HIV (264), which may lead to HIV-associated nephropathy, a disease characterized by rapid decline in renal function, high proteinuria, and FSGS of the collapsing type (265). FSGS can be seen in hypertensive patients. In these cases, the mechanical stress put on podocytes through the increased intraglomerular pressure may lead to damage of podocytes. Indeed, cultured podocytes have been found to be stress-responsive (266). Protein overload also leads to the development of FSGS (267,268). Consequently, not only damage to the podocyte, but also other mechanisms that cause increased passage of proteins through the glomerular filtration barrier may, via podocyte injury, lead to FSGS.

Despite this progress in the elucidation of the secondary FSGS, the etiology of the primary forms of FSGS remains elusive. Much research has been done to identify a putative humoral permeability factor. Such a factor would explain the high rate of FSGS recurrence in patients that receive a renal transplant because of FSGS. Also, a permeability factor would explain the transmission of a proteinuric condition from mother to her unborn child (269). Several groups, most notably that of Savin and coworkers, identified characteristics of this permeability factor, but the ultimate structure and origin remain unknown (262,270). Others have supposed that not the presence of a permeability factor, but the absence of crucial plasma factors lead to the development of proteinuria and FSGS. The hypotheses about the pathophysiologic mechanisms again incorporate the podocyte as a crucial target. Coward et al tested the influence of plasma of normal and nephrotic patients on the distribution and signaling of slit diaphragm proteins in cultured podocytes, and found that the effect of nephrotic plasma could be abrogated by adding normal plasma (271). Wei et al found that the plasma concentration of soluble urokinase receptor (uPAR) is elevated in serum of patients with recurrent FSGS (Wei et al, J Am Soc Nephrol 2008(19):103A). In mouse models, uPAR signaling in podocytes has been shown to cause foot process effacement and proteinuria (272), but mechanistical evidence for a pathogenic role of soluble uPAR in human FSGS is lacking.

Irrespective of the cause of proteinuria, podocyte injury is a crucial step in the further development of FSGS. Using combined observations from different animal models, Kriz (273) has described a sequence of events that explains the development of FSGS: podocyte injury leads to loss of podocytes, which results in denuded parts of the GBM and hypertrophy of the remaining podocytes. This will increase the possibilities for the formation of adhesions of the podocytes or the GBM to the parietal epithelial cells. Once such an adhesion has been formed, this leads to an encroachment of the parietal cells on the capillaries, and to 'misdirected filtration', the delivery of glomerular filtrate to the space between Bowman's capsule and the overlying parietal epithelial cells.

Despite the initial pivotal role of the podocyte, the further development of the FSGS lesions does involve other glomerular cell types. Kihara et al and Nagata et al suggested that in the collapsing and cellular variants of FSGS, the proliferating cells are of parietal origin (274,275). In a series of studies, Smeets et al and Dijkmans et al have recently brought more evidence for the involvement of parietal epithelial cells in the development of collapsing FSGS in animal models (35), human idiopathic FSGS (38), as well as HIV and pamidronate associated collapsing FSGS (39).

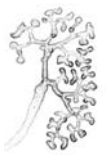
Taken together, the pathogenetic mechanisms in secondary FSGS all converge on podocyte damage as a central initiating step of development of proteinuria. In primary causes of FSGS, the initial pathogenetic mechanisms of proteinuria remain unclear, although a contribution of the podocyte is to be expected, and certainly this cell is at play in the later development of FSGS.

Diabetic nephropathy

Diabetic nephropathy is a complication of both type I and type II diabetes, and is currently the main cause of end stage renal diseases. Clinically, diabetic nephropathy is characterized by a decline in renal function that is preceded or accompanied by the development of proteinuria. Microalbuminuria is an important risk factor for the progression of the disease, and is currently used to screen patients at risk for development of diabetic nephropathy (276).

The histopathologic features of diabetic nephropathy comprise glomerular hypertrophy, thickening of the GBM and mesangial expansion in early stages of the disease. Later phases are characterized by the presence of glomerulosclerosis, sometimes showing the pathognomonic Kimmelstiel-Wilson nodules, together with interstitial fibrosis.

Mesangial cells as well as podocytes have been implicated in development of glomerulosclerosis in diabetic nephropathy, and especially cytokines and growth factors, such as TGF-β, connective tissue growth factor (CTGF), and insuline like growth factor have been extensively studied. With regard to the proteinuria in diabetic nephropathy, different components of the glomerular filtration barrier have received attention, and all seem to be affected by the diabetic condition. The thickening of the GBM may relate to a dysregulated assembly of matrix components, which translates into dysfunction. The endothelial cells are damaged in the diabetic milieu, through both metabolic and hemodynamic factors (277). In recent years, most attention has been given to the role of podocyte injury and podocyte loss in diabetic nephropathy. The attention for the role of the podocyte has been fueled by the seminal observation by Pagtalunan et al (278), that in Pima Indians with type II diabetes glomeruli show a marked decrease in podocyte number, a feature now called 'podocytopenia'. Podocyte damage in diabetic nephropathy can be induced by different factors, including high glucose per se (279), oxidative stress, the altered glomerular hemodynamic conditions (280), advanced glycation end products (281), growth factors such as TGF-β (279), and an increased activity of the local renin-angiotensin system (282,283). The



podocytes may subsequently be lost through different mechanisms. For example, increased TGF-β signaling has been linked to podocyte apoptosis (124). Also, the adhesion of podocytes to the GBM may be diminished as a result of a decreased integrin expression (284). These loosely attached podocytes may shed into the urine, and podocytes, as well as podocyte proteins, can be recovered from urine of diabetic patients (285,286). Furthermore, the podocyte may show functional deficits, as is exemplified by a decreased nephrin expression and foot process effacement. Many of the deleterious factors in the diabetic milieu affect the podocyte through stimulation of local angiotensin II production. Stimulating podocytes with angiotensin II in vitro leads to upregulation of TGF-β, providing a link to podocyte apoptosis (287). The effect of angiotensin II on the expression of nephrin and other slit diaphragm-proteins has been studied. A decreased immunostaining for these proteins in the presence of elevated angiotensin II levels has been described (283,288,289). These findings also explain the notion that in diabetic nephropathy angiotensin converting enzymes and angiotensin II inhibitors have a beneficial effect that cannot be explained solely by systemic blood pressure normalization.

Through podocyte loss, mechanisms are set in motion that further aggravate the proteinuria and glomerular damage. For example, Baelde et al (126) suggested podocyte loss as the cause of decreased glomerular VEGF expression, and hypothesized that this may infringe on the endothelial maintenance.

Membranous nephropathy

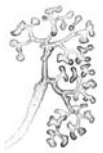
Membranous nephropathy is characterized by its histopathologic presentation, namely deposition of subepithelial immune complexes along the GBM in a granular fashion, without inflammation. In response to this, the podocyte forms and deposits GBM components between these immune complexes, visible as 'spikes' in light microscopy. Proteinuria, usually in the nephrotic range, is the most frequent symptom. The clinical course is related to the severity of the proteinuria, with frequent progression to end-stage renal disease in high-proteinuric individuals. Although several diseases, including malignancies, are associated with the development of membranous nephropathy, most cases are idiopathic.

Most of the knowledge about the pathogenetic mechanisms of proteinuria in membranous nephropathy has been derived from studies in an animal model that closely mimics this disease, Heymann nephritis (290). In this model, antibodies directed against the rat podocyte protein megalin (see above) give rise to the formation of immune complexes at the podocyte basal membrane that are subsequently shed and deposited in the lamina rara externa of the GBM. Here, these immune complexes activate complement, and the C5b-9 complex causes damage to the podocyte. Apart from causing direct damage, the C5b-9 complex also activates podocytes, resulting in the formation and release of proteases and reactive oxygen species (146,291). These components

damage the GBM as well as the podocyte itself through lipid and protein peroxidation. Furthermore, there are several intracellular consequences that result from the damage of podocytes by complement, including DNA damage (292) and cytoskeletal reorganization (293). The latter also influences the expression and distribution of nephrin and podocin (294,295), and presumably alters the function of the slit diaphragm. The activated podocytes also increase their production of GBM components, but it has been suggested that the assembly of this extracellular matrix is abnormal (171,296). Consequently, the proteinuria that is seen in the Heymann nephritis model has been explained by 1) changes in the GBM through abnormal assembly, increased breakdown, and damage through reactive oxygen species; and 2) damage to the podocyte, diminishing its adhesive properties to the GBM, disrupting the organization of the slit diaphragm, increasing podocyte DNA damage and apoptosis.

It is likely that in human membranous nephropathy, similar pathogenetic mechanisms are at play. Indeed, the role of reactive oxygen species has been suggested by a small clinical study that showed beneficial effects of the antioxidant probucol in patients with membranous nephropathy (297). However, in most cases, the antigen responsible for the development of this sequence of events is still elusive. The one exception is the antigen that is responsible for a rare congenital form of the disease, described by the group of Ronco in 2002 (148). They found that antenatal membranous nephropathy is caused by antibodies directed against neutral endopeptidase (NEP), an enzyme expressed at the podocyte membrane but also in the placenta. Anti-NEP antibodies developed in mothers that were NEP-deficient due to a mutation in the MME gene, but were exposed to the protein of fetal origin during pregnancy. In a subsequent pregnancy, the antibodies could cross the placenta and induce the disease (147,148). Recently, a preliminary report suggested that the phospholipase A2 receptor could be the target antigen in a subset of patients with membranous nephropathy (Beck et al, J Am Soc Nephrol 2008(19):104A).

If the pathogenetic mechanisms that have been revealed in animal models would also apply to humans, this could be of particular interest in patients that have developed membranous nephropathy secondary to a malignancy. These patients have apparently developed antibodies directed against components of the tumor that also react with normal components of the glomerulus. In the glomerulus, these antibodies are able to activate complement, thus it could be suspected that the same antibodies could have anti-tumor effects. It would be of interest to evaluate the clinical course of patients that have developed membranous nephropathy in comparison to those with the same type of cancer, but without membranous nephropathy development.



Consequences of proteinuria

Effects of proteinuria on the glomerulus

The effects of proteinuria on the glomerulus are often difficult to distinguish from causes of proteinuria. Injury to podocytes may, as discussed above, lead to proteinuria, but also one might envision harmful effects of increased protein passage on podocytes. Adding to this difficulty is the notion that factors that give rise to proteinuria may in addition lead to histopathologic changes, but without a causal relation between the two. A disturbed GBM make-up, for example, may lead to proteinuria and podocyte changes independently.

Cause and effect discussions aside, there are some clear histopathologic observations in proteinuria, the most consistent being that proteinuria is almost always accompanied by profound changes in podocyte morphology. The highly organized cellular podocyte architecture is lost, transforming the podocyte into a flattened, simplified epithelial cell. Effacement of foot processes takes place through widening and shortening, and at the base of the flattened foot processes, a dense band of actin filaments is seen (298). Podocyte hypertrophy is often observed, together with microvillous transformation and pseudocyst formation. Also, protein reabsorption droplets that contain plasma proteins are seen in the podocyte cytoplasm. There is often an increased or renewed expression of mesenchymal – and thus for podocytes embryonic – markers, and according to Kerjaschki it may be concluded that in proteinuria 'podocytes recapitulate their development in reverse' (50). There is some discussion as to whether the extent of foot process effacement is related to the amount of proteinuria, with some studies indicating a positive relation (259,298), while others did not find such a correlation (299). Foot process effacement influences the ultrafiltration coefficient, and there is a correlation between the amount of foot process effacement and the GFR (218). Consequently, according to Smithies, the decrease in GFR may result in proteinuria (212). In most cases, however, podocyte foot process effacement seems to accompany the development of proteinuria, sometimes lagging behind.

Abbate et al studied the changes in podocytes in a rat model of renal mass reduction, and described that the changes in the podocytes related to the amount of protein accumulation in podocytes in vivo, and that anti-proteinuric therapies proved to be helpful. In vitro, stimulation of podocytes with albumin led to an increases production of TGF-β (267). In a follow-up study, the same group found that protein overload led to the upregulation of the noxious molecule endothelin-1 via dysregulation of the podocyte cytoskeleton (53). In this way, proteinuria per se may be the podocyte-damaging factor that leads to loss of podocytes, and to a sequence of changes as described before under focal and segmental glomerulosclerosis (300).

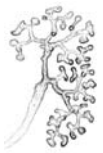
Effect of proteinuria on the tubulointerstitial compartment

The pathophysiology of the consequences of proteinuria for the tubulointerstitial compartment has been more clearly delineated. Again, it has been difficult to indicate to what extent the amount of proteinuria is a marker of the damage, instead of the actual cause of it (301). This question has been addressed using the amphibian axolotl as a model system. Some nephrons in the kidneys of these amphibians are connected to the peritoneal cavity, while others are closed. Gross et al found that upon injection of fetal bovine serum in the peritoneal cavity of these animals, only the tubular epithelial cells of the nephrons with a connection to the peritoneal cavity were activated and showed interstitial fibrosis (302). This suggests a causal role for increased protein passage in tubulointerstitium injury, and supports hypotheses based on more correlative evidence.

Several mechanisms may be responsible for the toxic effect of proteinuria on tubules. For example, protein cast formation may lead to obstruction of tubuli (301), filtered proteins such as complement factors may be toxic to the tubular epithelial cells, and cytokines may directly activate the tubular epithelium (303). Most attention has been given to uptake of proteins by tubular epithelial cells. During proteinuria, multiple macromolecular components reach the apical side of the tubules. Which one of these is harmful is a matter of discussion. Below is a description of the factors that are held responsible for the toxic effects of proteinuria on the tubulointerstitial compartment, and the molecular pathways through which such an effect is mediated.

The toxicity of albumin has received a lot of attention. In vitro, incubation of tubular epithelial cells with albumin has a profound effect: the endocytosed albumin, either directly or via the protein kinase C-dependent formation of reactive oxygen species (304), activates several signaling proteins, including mitogen-activated protein kinases (305), nuclear factor κB (306,307), and signal transducer and activator of transcription proteins (308). This activation of tubular epithelial cells results in the modulation of cytokine production and ECM regulation: albumin upregulates the tubular expression of interleukin-8 (307), macrophage chemoattractant protein-1 (306), RANTES (309), and endothelin-1 (310); it increases the expression of different collagens (311), profibrotic cytokines such as TGF-β (312), and tissue inhibitors of metalloproteinases (TIMPs) (313). Also tubular production of angiotensin II is increased (314). These changes lead to an interstitial inflammatory response, and promote the development of interstitial fibrosis. Furthermore, protein overload has been shown to directly lead to increased tubular apoptosis (315).

However, there are reports that failed to show a direct effect of albumin on tubular epithelial cells (316). For example, Burton et al found that although plasma had a clear effect on cytokine production and ECM regulation, purified albumin failed to induce this effect (317,318). This suggested that the substances bound to albumin, but not albumin itself, promotes the aforemen-



tioned cellular changes. Indeed, several experiments have shown an effect of albumin-bound free fatty acids (319,320).

Proteinuric Nagase analbuminaemic rats do develop tubulointerstitial fibrosis in the absence of albumin (321). This, together with other observations, has suggested that other proteins may also be responsible for the toxic effect of proteinuria on the tubular epithelial cells. These include transferrin, immunoglobulins, cytokines, growth factors, and complement (301). For example in membranous nephropathy, the C5b-9 complex may bind to the tubular apical surface and damage these cells (293). Also, filtered complement components may be activated locally via the alternative pathway, and tubular epithelial cells also synthesize complement in response to protein overload (322).

Most of the insight into the consequences of exposure of tubular epithelial cells to increased amounts of plasma proteins has been derived from in vitro studies. These studies have been criticized because they use protein concentrations that are outside the physiologic range. However, as stressed by Remuzzi (323), the protein concentration in vivo may be low in Bowman's space, but increases as the pre-urine passes along the nephron and water is absorbed. Also the duration of the in vivo exposure is usually longer than in cultured cells.

Taken together, several components that are present in excessive amounts during proteinuria seem to act on the tubular epithelial cells, thereby creating a pro-inflammatory and pro-fibrotic milieu. Together with tubular atrophy and apoptosis, interstitial inflammation and fibrosis form the histopathological representation of progressive renal diseases, for which mechanisms are discussed in a later paragraph.

Cardiovascular risk

The nephrotic syndrome comes with changes in the blood lipid profile (hyperlipidemia and hypercholesterolemia). Such changes are related to the development of atherosclerosis. However, also low levels of albuminuria, even in the high normal range, increase the risk for cardiovascular disease. How can the effect of microalbuminuria on the cardiovascular system, most notably atherosclerosis, be explained at a pathophysiological level? This is a question that remains largely unanswered. In a review on the different possibilities to explain the clear epidemiologic link between the two, Stehouwer and Smulders (324) propose that atherosclerosis and microalbuminuria may be the result of a common pathophysiologic process, such as endothelial dysfunction. This hypothesis, modified from the Steno hypothesis that places more emphasis on systemic changes in basement membrane composition (325), is of interest in the context of an increasing appreciation of the importance of the endothelium in the permselectivity of the glomerulus. Another pathophysiological explanation is that mutations in genes that cause proteinuria also affect the heart, as may be the case with NPHS2 (326). However, it is unlikely that this explains

the cardiovascular risk in the majority of patients that do not have similar mutations. Furthermore, a systemic proinflammatory state associated with nephrotic syndrome may provide a link to cardiovascular changes (327).

Progression of renal disease

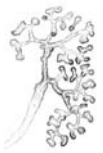
The kidney has a stereotypic reaction to injury

Regardless of the initial cause of renal disease, the histopathologic lesions that parallel the decline in renal function include glomerulosclerosis, tubular atrophy, interstitial fibrosis, and interstitial inflammation. This has given rise to the widely accepted notion that the progression of renal damage involves a common mechanism. Ideas about the nature of this mechanism and, accordingly, the temporal relationship of the histopathological lesions (e.g., does the inflammation precede or follow the development of fibrosis) vary, as discussed below in more detail.

Next to common histopathological features, the different renal diseases, both in native and transplant nephropathies, share risk factors for progression. These include proteinuria and hypertension. Proteinuria as a risk factor for native kidney diseases has already been discussed. Proteinuria has a comparable detrimental effect in the transplantation setting. Several groups found that even low grade proteinuria in renal transplant patients is an independent predictor of graft loss, and anti-proteinuric therapies have a beneficial effect on graft survival (328-330). Zayas et al measured dextran sieving coefficients in allografts and found that even in well-functioning grafts, there is some loss of glomerular permselectivity (331), and suggested a link between this glomerular leakiness and progression of transplant pathology.

Hypertension represents another risk factor for progression of both native and transplant renal disease (332). Haroun et al found a relationship between the severity of hypertension and the risk to develop end stage renal disease (333). This is also true in the transplantation setting (334). Proteinuria and hypertension seem to amplify each others effect (330,332). The pathophysiologic explanation for this interaction will be outlined below.

Of all the histopathological characteristics of progressive kidney disease, the lesions that correlate most closely with renal function loss are those found in the interstitium (335,336). Interestingly, a substantial portion of renal disease primarily affects glomeruli. The link between the damage in these two tissue compartments and the role of the mentioned risk factors therein will be described in the following paragraphs.



How does glomerular injury lead to tubular damage

There are several theories about the mechanisms that connect glomerular damage to tubular injury and interstitial fibrosis (303,336,337).

1) In a previous paragraph, the hypothesis that proteinuria has a central role in the connection between glomerular and tubular injury has been discussed. This so-called Remuzzi theory attributes a toxic effect of the increased amount of proteins filtered by the glomerulus on the tubular epithelial cells, resulting in a cellular reaction that promotes influx of inflammatory cells and fibrogenesis (234,235,322).

2) In a series of studies, Kriz has lined out another sequence of events that connects glomerular damage to tubular injury and nephron loss (303,338-340). The starting point is damage to or loss of podocytes that predisposes for the formation of an adhesion of the naked GBM to Bowman's capsule. Capillaries in these adhesions may still be patent, at least for some time, leading to filtration of plasma products into the space between the parietal epithelial cell and their basement membrane – so-called misdirected filtration. This leads to the formation of a proteinaceous crescent that, if misdirected filtration persists, spreads over a large part of the glomerular circumference and continuously into the tubular compartment, separating the tubular epithelial cells from their basement membrane (338). This leads to atrophy of the proximal part of the tubule or even complete obstruction of the tubular lumen, resulting in collapse of the tubule and further atrophy downstream. Tubular atrophy is followed by apoptosis of tubular epithelial cells, removal by the infiltrating mononuclear cells, and scar formation, ie, interstitial fibrosis.

Damage remains confined to the individual nephron involved in the process of misdirected filtration – proximal tubular atrophy and obstruction – tubular decomposition and scar formation. This is in seeming contradiction with the notion that loss of renal function is related to tubulointerstitial changes, and not to glomerular injury. Kriz and co-workers explain this by pointing out that the nephron disappears completely, leaving a fibrotic nephron recognized as a tubulointerstitial scar, but including a sclerotic glomerulus (338).

3) The glomerular cytokine theory (341) is yet another explanation of the link between tubular injury and glomerular damage. The theory applies most directly to inflammatory glomerular diseases. Three phases are distinghuished: i. delivery of cytokines and growth factors expressed in the glomerulus via diverse routes to the tubulointerstitial compartment, stimulating local interstitial and tubular cells; ii. stimulated cells express further chemotactic factors that attract mononuclear cells; iii. these cells release growth factors that mediate further injury to the tubules, and promote interstitial fibrosis.

4) Another concept of the etiology of chronic kidney disease focuses on the role of vascular changes and ischemia (342). Indeed, areas of tubular atrophy and interstitial fibrosis show a

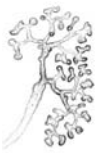
decreased density (rarefaction) of peritubular capillaries. In these areas, there seems to be an impaired angiogenic balance, with a predominance of antiangiogenic factors such as thrombospondin-1 and a decrease of angiogenic factors such as nitric oxide and VEGF (342,343). The resulting ischemia drives the development of interstitial fibrosis, which in turn results in an even more impaired delivery of nutrients to the tubular epithelial cells, thus perpetuating the process. Conversily, injection of pro-angiogenic compounds such as VEGF slow the development of interstitial fibrosis (344). Also, hypoxia promotes inflammation, resulting in further tissue damage and fibrosis.

While these observations clearly point at the importance of the microvasculature in chronic renal diseases, the way in which glomerular damage leads to ischemia and tubulointerstitial microvascular changes is less clear (303). One might envision an impaired blood flow as a result of glomerular capillary damage (345). Furthermore, glomerular production of angiotensin II may cause vasoconstriction in the efferent arterioles, resulting in a diminished blood flow into the peritubular capillaries. Also, pro- and anti-angiogenic factors expressed in the glomerulus may reach the tubular compartment via the normal blood flow; a disturbance in the glomerular expression pattern of pro- and anti-angiogenic factors may thus translate to a shift in the tubulointerstitial angiogenic balance. In this regard, it is of interest that Baelde et al (126) found a negative correlation between glomerular VEGF production and interstitial fibrosis. However, the exact nature of the mechanisms that link glomerular changes to interstitial vasculature deserves further study.

5) Related to the previous mechanism of interstitial fibrosis is the hypothesis that, as nephrons are lost during disease progression, the remaining nephrons start to hyperfunction, thereby increasing their metabolic demands (336). This will impact on their oxygen consumption and even in the presence of a normal microvasculature this may lead to hypoxia (345). Another consequence of the increased workload of tubular epithelial cells may be the increased formation of ammonia, which in turn can activate complement via the alternative pathway, favoring an inflammatory response (346,347).

These pathways are not mutually exclusive, and several pathways may be at play simultaneously and in an additive fashion. For example, interstitial fibrosis as a result of tubular activation will aggravate tubular epithelial hypoxia; tubulotoxic effects of proteinuria induce expression of vasoconstrictive molecules and evoke inflammatory responses that suppress angiogenic factors, leading to further ischemia.

Kriz' misdirected filtration theory is the most dissimilar of other pathways, although misdirected filtration does not rule out a concomitant role for excess protein trafficking. The misdirected filtration theory is especially applicable in diseases that have a glomerular cause, while the other pathways listed also apply to situations in which the tubulointerstitium is primarily targeted, for example in the case of transplant rejection. The most crucial difference between the misdirected



filtration theory and the other theories listed relates to the role that interstitial fibrosis is assumed to have. From a teleological point of view, Kriz et al (303) argue that the inflammation clears the remnants of an already irreversibly damaged nephron, which is subsequently replaced by fibrotic material. This limits the damage to a single nephron and at the same time retains the architecture of the remaining tissue. A common theme in the other pathways is the attraction of inflammatory cells to functional, albeit damaged, nephrons that orchestrate the development of interstitial fibrosis. In this scenario interstitial fibrosis is assumed to be a harmful event leading to further interstitial damage and loss of functional tissue. The molecular mechanisms that underlie development of such inflammation and fibrosis will be discussed next.

Mechanisms of interstitial fibrosis

Inflammation

As a result of the injuring mechanisms lined out above, tubular epithelial cells become activated and express pro-inflammatory molecules. MHC class II expression on tubular epithelial cells is upregulated, adhesion molecules including osteopontin and VCAM are expressed, and cytokines and chemokines including MCP1, RANTES, fractalkine, endothelin 1, and IL-6 and IL-8 are released (348,349). This is generally seen as the starting point of further damage: The pro-inflammatory milieu attracts leukocytes, primarily monocytes/macrophages and T-lymphocytes. These in turn secrete factors such as IL-1, interferon γ, and TNF-α which further activate tubular epithelial cells, thus initiating a self-perpetuating process of injury and inflammation.

Cells that contribute to fibrosis; EMT

Injury, inflammation, and ischemia are at the basis of fibrosis: this milieu promotes fibrogenesis by different cells. Tubular epithelial cells may increase their production of ECM, and also macrophages are thought to contribute to the interstitial fibrosis, although they additionally regulate matrix degradation. The cells that are mainly responsible for the deposition of the ECM are fibroblasts. There is some controversy as to what the main source of these fibroblasts is. Increase in the number of fibroblasts in the tissue results from the proliferation of local fibroblasts. A small percentage probably originates from the circulation. Furthermore, fibroblasts may derive from the tubular epithelial cells through a process of epithelial-mesenchymal transformation (EMT), a process that has received much attention in recent years. During EMT, epithelial cells lose typical epithelial characteristics (intracellular adhesions, cellular polarity, differentiation markers), gain features of mesenchymal cells (cell motility, expression of fibroblast markers such as FSP1/S100A4), and migrate to the interstitium. In mouse models of progressive fibrosis, EMT was an important source of interstitial fibroblasts, responsible for at least 36 percent of these cells (350). Although such a cellular plasticity may seem striking at first, it should be remembered that tubular epithelial cells derive from mesenchymal cells, making EMT a known pathway traveled in the opposite direction

(351). Others have disputed the importance of EMT, pointing at the fact that tubular epithelial cells in transit have up until now never been observed (352). Irrespective of the source of fibroblasts, the inflammatory milieu in the interstitium promotes their proliferation, activation, and ECM production. Activated fibroblasts are characterized by the expression of α-smooth muscle actin, although not all fibroblasts that contribute to fibrosis express this marker (353).

The signaling pathways that promote fibrogenesis have been investigated, but will not be extensively covered here. One of the best studied proteins in this respect is TGF-β, a multifunctional cytokine that has a central role in interstitial fibrosis. Indeed, TGF-β increases the production of ECM components by tubular epithelial cells and fibroblasts. Also, TGF-β, together with other cytokines such as basic fibroblast growth factor 2 (FGF-2), is an important inducer of EMT (2).

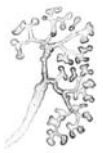
ECM composition and regulation

As described in paragraph, under normal conditions collagen IV is present in the tubular basement membrane. During interstitial fibrosis tubular epithelial cells also express other types of ECM molecules such as fibronectin, and collagens I and III. Fibroblasts express these ECM molecules, and also laminin and tenascin.

Accumulation of ECM is the result of an imbalance between synthesis and repair. The factors that determine this balance have been mapped in some detail. Matrix metalloproteinases degrade ECM components, and may thus contribute to the resolution of fibrosis. The activity of MMPs is regulated by, among other factors, tissue inhibitors of metalloproteinases (TIMPs), indicating that the synthesis and degradation-balance is regulated at multiple levels. Adding further complexity to the system is the fact that the role these molecules play is in part dependent on their spatial expression pattern. For example, MMP2 may have a beneficial effect in degradation of interstitial fibrotic material, but at the other hand may promote the process of EMT by disruption of the tubular basement membrane (2,353).

Damage is progressive

After loss of a critical amount of nephrons loss of kidney function is progressive. The widely tested and accepted mechanism for this progression is explained by the hyperfiltration hypothesis, as put forward by Brenner and Hostetter and co-workers in the 1980s (354,355). This hypothesis states that as nephrons are lost, an adaptive process is initiated that leads to increased intraglomerular pressure and flow across the glomerular capillary wall. These two factors increase the single nephron glomerular filtration rate. While these adaptations limit the total loss of renal function (as determined by the GFR), they are eventually detrimental. In rat models a severe reduction in renal mass brought about hemodynamic and histopathologic changes within one week (355).



The mechanisms that regulate the initial adaptive hemodynamic changes are incompletely understood. Also, several factors may be held responsible for the further damage of the glomerulus, including mechanical stress due to increased intraglomerular pressure that disrupts vascular integrity or leads to podocyte damage. Increased protein trafficking has also been suggested to directly damage podocytes. These uncertainties at the molecular level aside, it is clear that at the tissue level the adaptive response to a loss of nephrons has an adverse effect that potentially results in glomerular damage, leading to further nephron loss along pathways lined out before, thus resulting in a self-perpetuating loss of renal function.

As a result of this progressive course of kidney diseases, patients suffering from these diseases will eventually need renal replacement therapy. One of these therapies, kidney transplantation, will be discussed in the next paragraphs.

PART 3 | LONG-TERM DYSFUNCTION OF KIDNEY TRANSPLANTS

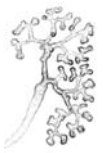
In the last 40 years, transplantation has become the treatment of choice for end-stage kidney failure. In 1954, the first successful kidney transplantation was performed by dr. Joseph Murray, who was later awarded the Nobel Prize for this achievement. In the absence of rejection – the transplanted kidney was donated by the twin brother of the recipient – this operation showed the technical feasibility of transplantation. Developments in immunosuppression, starting with prednisone and azathioprine, made non-HLA identical transplantation possible, although rejection severely limited graft survival. In these early days of kidney transplantation the first year graft survival rate was around 50 percent (356). The application of the newly discovered immunosuppressive drug Cyclosporine A in 1978 (357) proved to be a breakthrough in transplantation. This immunosuppressive drug, together with improvements in donor matching and storage condition of the graft, provided the basis for the current successful use of kidney transplantation as a treatment of end-stage renal disease. Nowadays, the factors that limit the success of transplantation have shifted from the acute to the chronic phase after transplantation. Long-term failure of kidney transplants, chronic allograft dysfunction, is the main reason of allograft loss.

This paragraph describes the clinical and histopathological characteristics, pathophysiologic mechanisms, and diagnostic molecular markers of chronic allograft dysfunction.

General description and definitions

Limited improvement in long-term survival

The introduction of current immunosuppression including calcineurin inhibitors and mycophenolate mophetil fostered an increase in short-term graft survival. Currently, the first year renal transplant survival rate is about 90 to 95 percent. In contrast, long-term graft survival has not made similar progress. The long-term attrition rate has even remained fairly constant over the past 25 years, with a half-life of cadaveric transplants of about 8 years in the United States (358-360). Thus, for patients with a kidney transplant the most threatening problems are those that arise on the long-term, with chronic allograft dysfunction and death with a functioning graft being the two leading causes of graft loss (359,361,362). Intriguingly, diverse and seemingly opposed processes may contribute to this long-term attrition of allografts, including ongoing immunologi-



cal activity or 'chronic rejection', as well as toxicity of immunosuppressive medication, meant to prevent chronic rejection.

Definitions of long-term graft failure

Over the years ideas about the pathophysiological mechanisms leading to long-term allograft failure have changed, and description of the clinical and histopathological presentation has varied. This has led to some unclarity in definition of the subject, with terms as chronic allograft/transplant dysfunction, chronic allograft nephropathy, and chronic rejection used interchangeably to describe the same problem. In this paragraph, the following definitions are used:

Chronic allograft dysfunction describes the clinical aspects of long-term renal allograft failure. The clinical features are not specific for one diagnosis, and chronic allograft dysfunction should thus be regarded as a clinical syndrome that can be brought about by different and mixed pathophysiological processes (363). Its pleomorphic etiology is in part reflected by the elaborate list of risk factors.

Chronic allograft nephropathy (CAN) is the term used to describe the histopathological changes seen in biopsies from patients with chronic allograft dysfunction. Again, these histopathological changes are not specific for one diagnosis, and in this definition CAN is the histopathological representation of the spectrum of causes that lead to chronic allograft dysfunction. In biopsies with features compatible with only one diagnosis the term CAN should be avoided (364). Chronic rejection, for example, is a distinct diagnosis. It denotes a central role for immunological processes as the cause of chronic allograft dysfunction, and can be differentiated from other causes of allograft functions by certain discriminatory features in the renal biopsy (365).

This paragraph will describe chronic allograft dysfunction and its risk factors, chronic allograft nephropathy, and the pathophysiological processes involved in these two syndromes, with a special focus on chronic rejection and chronic calcineurin inhibitor toxicity.

Chronic allograft dysfunction

Clinical manifestations

Chronic allograft dysfunction is characterized by a slow but progressive decline in renal function starting after the first three months post transplantion. This is often combined with aggravation or de novo development of hypertension and proteinuria (366,367). The time to eventual complete loss of renal function depends on the initial graft function (intercept) and rate of progression

(361). The decline in GFR in patients that have survived the first year post transplantation varies between 1.2 and 2.5 ml/min per year (368).

Scope of the problem

Once chronic allograft dysfunction has started to develop, the process will invariably progress to end stage allograft failure. Chronic allograft dysfunction is the main cause of returning to dialysis after transplantation (369). Currently, patients waiting for a repeat transplant make up about 15 to 20 percent of the total number of patients on the waiting list for renal transplantation (www.transplantatiestichting.nl, www.optn.org). Retransplantation carries an additional risk to develop chronic allograft dysfunction (370).

Risk factors

The development of chronic allograft dysfunction is influenced by a plethora of factors, both immunological and non-immunological, and related to donor and recipient. The intuitive bottom-line, as stated by Paul in a review on the subject, is that chronic allograft dysfunction 'seems to develop in kidneys from older donors or kidneys that have acquired damage later on' (371). The most important risk factors are listed below, grouped as immunological and non-immunological factors. It should be noted that grouping risk factors is artificial, as it is their combined and often synergistic effects that modulate graft survival. Indeed, different forms of primarily non-immunological damage directly affect graft function, and in addition modulate the immunogenicity of the tissue (372).

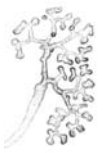
Immunological risk factors

Histoincompatibility

Evaluation of large series of renal transplants in the US (373), Europe (374), and the UK (375) has made clear that human leukocyte antigen (HLA) mismatches have a detrimental effect on long-term allograft survival. In a US series, the 10 year graft survival was 52 percent in HLA-matched and 37 percent in HLA-mismatched transplants (373). Mismatches in the different HLA loci (HLA-A, B, DR) seem to have a comparable impact on graft function, at least on the long-term (376). The importance of HLA matching for long-term allograft survival may be declining (377,378). Indeed, in comparison to risk factors such as donor age, the effect of HLA mismatches is nowadays relatively small (368).

Sensitization and Panel Reactive Antibodies

Blood transfusions, pregnancies, and previous transplantations may give rise to the formation of anti-HLA antibodies. The presence of such antibodies is tested by evaluating the reactivity of the serum of a potential transplant patient with a 'panel' of lymphocytes obtained from selected



blood donors. The number of donor samples in the panel with which the patients' serum reacts is an indication of the patients' extent of presensitization. The amount of 'panel reactive antibodies' (PRA) present before transplantation and the formation of such antibodies after transplantation is related to the development of chronic allograft dysfunction (363,379-381).

Immunosuppression and non-compliance

Improvements in immunosuppression have been instrumental in reducing the number of acute rejections but not the long-term complications. Non-compliance to immunosuppressive drugs may be one of the factors explaining this discrepancy (361,382). In a meta-analysis the odds ratio of graft loss in non-compliant versus compliant patients was 7.1 (382).

Acute rejection

HLA-mismatches, presensitization, and non-compliance all relate to what is the most important immunological risk factor of chronic allograft dysfunction: acute rejection (363,383). Progress in graft survival has been less pronounced in patients that have experienced acute rejection episodes (362). The effect on long-term allograft function varies with the number, severity, timing, type, and response to treatment of acute rejection (369,384,385). When adequately treated, early acute rejection (within 3 months after transplantation) does not (386,387), or to a lesser extent (388,389), affect long-term outcome when compared to late acute rejections. The effect of vascular rejection is worse than that of interstitial rejection (390).

At the same time, improvements in treatment of acute rejection have not translated into better survival on the long-term (360). This may indicate that only the less harmful acute rejections are treated sufficiently, and also points towards the influence of other risk factors that may coexist or interfere (386). For example, it has been suggested that while cyclosporine accurately prevents acute rejection, it also inhibits development of tolerance (391,392). Additionally, nephrotoxic adverse effects of immunosuppressive medication such as cyclosporine may explain the lack of enhanced long-term graft survival over the years.

Non-immunological risk factors

Elegant studies by Gourishankar et al showed that the function of a renal allograft could be predicted by the function of its 'mate graft' – the other graft from the same donor (393). Even though rejection was not influenced by this so-called 'mate effect', long-term outcome was paired even up to 8 years after transplantation (368). This suggests that donor-related factors have an important role in transplantation.

Donor age and renal mass

Donor age is an important risk factor for long-term graft loss (383,394-396), and explains 30 percent of the variation in transplant outcome after five years (366,397). The influence of donor age on graft survival can in part be explained by immunological mechanisms. Donor age modulates the occurrence of rejection and subsequent tissue repair: older kidneys seem to be

more immunogenic and have impaired repair mechanisms (398). However, it is unlikely that this increased immunogenicity completely explains the effect of donor age, as donor age also influences survival of zero HLA-mismatch transplantations (394).

A risk factor related to donor age is renal mass: small kidney size in relation to the recipient is related to adverse outcome (399), although this may not be true in a pediatric setting (400). Likewise, male donor gender seems to convey a benefit in long-term graft survival, which is probably also related to renal mass (368).

Recipient age and sex also have a clear influence on allograft survival, explaining 10-25 percent of the variation of the long-term outcome (397), with female sex and younger age having a better prognosis (401). In the long-term, the effects of donor and recipient age are synergistic (402).

Peritransplant injuries

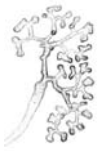
The donation and transplantation procedures have influence on the long-term function of renal allografts. Risk factors include brain death, preservation of the allograft, and ischemia/reperfusion injury. Terasaki et al (403) studied the three-year survival rates of cadaveric donors, living related donor, and unrelated donor grafts (e.g. spouses). They found that survival rates were similar between living related and living unrelated donors, despite a higher number of HLA mismatches in the latter. Both living donor groups showed a higher survival than cadaveric donors (403). This shows the importance of brain death, preservation, and ischemia/reperfusion injury in determining survival on the long-term. Through which mechanisms these factors influence graft function is not completely known. Again, an interaction between non-immunological and immunological mechanisms is to be expected. Koo et al demonstrated an increased expression of tubular antigens and inflammatory molecules in biopsies from cadaveric donor kidneys in comparison to living donor kidneys. This was associated with the occurrence of acute rejection episodes (404).

Delayed graft function

Delayed graft function is a form of acute renal failure, mostly defined as the requirement of dialysis early after transplantation. According to some studies delayed graft function is an independent predictor of late allograft function (405-407). Others attribute the effects of delayed graft function to the influences on the occurrence of acute rejections, for example through an increased immunogenicity of the graft (367). Indeed, acute rejection in the setting of delayed graft function has an additive adverse effect on graft function (405,408). In itself, delayed graft function is the result of a number of risk factors, including some of those mentioned above (368,406,409).

Post transplant injuries

After transplantation, complications may arise that have a detrimental influence on long-term graft survival:



Proteinuria conveys an increased risk of renal allograft loss (328,329,383,386,398,410-413). Twenty to 28 percent of the patients with chronic allograft dysfunction have proteinuria, compared to six to eight percent of patients without this condition (386,414). In a study by Hohage et al, early development of proteinuria that lasted longer than 6 months was noted in a quarter of patients. The proteinuric patients had a 5 year survival rate of 59 percent, compared to 86 percent in the non-proteinuric patients (329).

Hypertension is also associated with increased chances of late graft failure (334,415), although the effect is less pronounced than that of proteinuria (412). Development of proteinuria seems to be linked to development of hypertension (330), and the presence of both conditions has an additional risk for allograft loss (410).

Immunosuppressive treatments contribute importantly to the development of chronic allograft dysfunction. The nephrotoxic effects of calcineurin inhibitors such as cyclosporine A and tacrolimus are well-recognized, but also corticosteroids have been found to contribute to chronic allograft dysfunction (366). The extent of their contribution is more difficult to estimate (369). The histopathologic changes that are related to calcineurin toxicity will be discussed in more detail in the following sections.

The pathology of chronic allograft dysfunction

Changing definitions

As pointed out before, concepts about pathophysiological mechanisms that lead to chronic renal allograft dysfunction have changed over the years. In the Banff 91 classification (see below), the term chronic allograft nephropathy was introduced to describe the histological changes seen in biopsies from patients with chronic allograft dysfunction that could not be related to one specific cause. These changes had previously been described as chronic rejection, but as this term implied a predominantly immunological mechanism of damage, the more neutral terms chronic allograft nephropathy (CAN) was chosen (364-366). Since then, definitions of CAN in the literature have been different, ranging from strict histopathological entities to loose descriptions of clinicopathological syndromes. It was increasingly noticed that the indiscriminate use of the term CAN would limit the possibilities of establishing an etiologic diagnosis, and would result in a term with 'little value other than to hide our ignorance' (364). Indeed, treatments for the different causes of CAN, once they could be distinguished, are likely to be fundamentally different (416). In order to

prevent further indiscriminate use of CAN, the latest iteration of the Banff classification has now moved towards elimation of the term CAN, replacing it by the category 'Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology'.

Because most of the studies described herein have used the term CAN to denote the changes seen in biopsies, we will also use it in the following paragraph. Here, CAN is defined as the histopathological changes seen in biopsies from patients with chronic allograft dysfunction without a recognizable single cause or diagnosis. In this definition, CAN is the histopathological representation of the spectrum of causes that lead to chronic allograft dysfunction. From a clinical point of view the most important differential diagnoses of CAN are chronic rejection, chronic calcineurin inhibitor toxicity (414), obstruction, and infection (365). In this paragraph, the histopathology of CAN, chronic rejection, and chronic calcineurin inhibitor toxicity will be described.

Causes and course of CAN

The factors that contribute to the development of CAN in allograft kidneys include ischemia, hyperfiltration, proteinuria, hypertension, de novo or recurrent glomerular disease, infection, drug toxicity, and (chronic) allo-immune injury (417). These different factors damage all compartments of the kidney (vasculature, tubules, interstitium, and glomeruli), though not always simultaneously or to the same extent. This often limits the possibilities to distinguish the contribution of different causes to the development of the lesions seen in CAN.

At 1 year post transplantation CAN is already widely present in renal allografts. In protocol biopsies the number of patients affected by CAN ranges from 50 to 94 percent (418-420). The largest of these per-protocol series, a study of 120 simultaneous kidney-pancreas transplants with over 950 biopsies, found that the development that CAN could be divided in two phases. The initial phase was characterized by interstitial damage and rapid development of tubular atrophy and interstitial fibrosis. CAN developed earlier in allografts that showed signs of peritransplant injury (acute tubular necrosis) and acute rejection (420). Also, subclinical rejection was related to earlier development of CAN, but this was not found in later studies specifically addressing this relation (421). The later phase of CAN was characterized by a more severe involvement of the vasculature and glomerulosclerosis (420).

Histopathologic features

The three main features of CAN are fibrous intimal thickening, interstitial fibrosis, and tubular atrophy.

Fibrous intimal thickening indicates the changes seen in renal arteries of allografts, and affects mostly the larger (arcuate) arteries. The normal intima consists of a single layer of endothelial cells, based on the internal lamina elastica. In CAN, the renal arteries show a concentric expansion

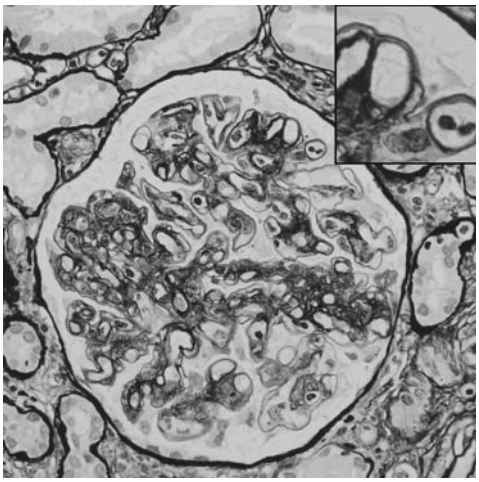


Figure 4. Transplant glomerulopathy. Transplant glomerulopathy is seen in chronic rejection. This glomerular lesion is characterized by a duplication of the glomerular basement membrane (inset).

of the intima, narrowing the arterial lumen. Although this is comparable to the changes found in normal atherosclerosis (369), the concentric nature of the lesion is somewhat specific for CAN. The presence of inflammatory cells in the neo-intima, as well as disruption of the internal elastica seems to be more specifically related to chronic rejection (see below). Smaller vessels and arterioles may also be affected in CAN, showing for example arteriolar hyalinosis. This lesion is often indicative of calcineurin inhibitor toxicity, and will be discussed below. Multilayering of the basement membrane of peritubular capillaries is another vascular feature of CAN. Severe multilayering is associated with chronic rejection (366).

Interstitial fibrosis and tubular atrophy often occur simultaneously. Atrophic tubules show a thick tubular basement membrane and small tubular epithelial cells. An aspecific inflammatory reaction is often found in conjunction with the atrophic tubules and interstitial fibrosis. The molecular composition of the interstitial fibrotic lesions will be further discussed below and in chapter 6 of this thesis.

Glomerular changes may also be seen in CAN, and include focal segmental as well as global glomerulosclerosis, and mesangial matrix expansion. Other lesions affecting the glomeruli include mesangiolysis and duplication of the GBM. This may again point to a specific etiology discussed below.

Since 1991, the Banff Working Classification of Renal Allograft Pathology ('Banff classification') has been used for standardization and grading of renal allograft biopsy interpretation. The Banff classification provides a grading for the individual CAN lesions (tubular atrophy, interstitial fibrosis, fibrous intimal thickening, mesangial changes, glomerulopathy). Because tubular atrophy and interstitial fibrosis are in general most accurately sampled, the extent of these changes is used for grading the severity of CAN (in grades I to III, corresponding to mild/moderate/severe) (422). The most recent iteration of the Banff classification has maintained this grading system, but now with respect to the category 'Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology' (365).

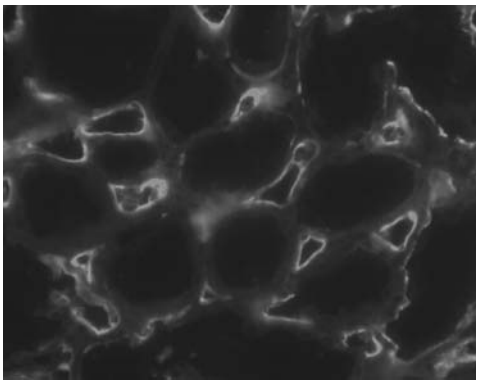


Figure 5. C4d deposition. In chronic humoral rejection, the complement split product C4d remains covalently bound to endothelial cells. This picture shows widespread deposition of C4d in peritubular capillaries.

Lesions related to specific etiology

As mentioned before, the mixture of pathophysiologic processes operating simultaneously leads to a histopathological picture that does not allow diagnosis of a single factor as the cause of the allograft dysfunction. In some cases, however, a single cause may be of predominant importance, and can be recognized by certain clinical and histological features. Two of these disease entities are described below: chronic rejection and chronic calcineurin inhibitor toxicity.

Chronic rejection

Chronic rejection is defined as injury caused by alloreactivity directed against the graft. Signs of both cellular and humoral forms of chronic rejection can be present in the renal allograft biopsy. The Banff classification denotes transplant vasculopathy, ie, disruption of the internal elastica, and infiltration of inflammatory cells in the thickened intima of the renal vasculature, as a sign of chronic rejection (422). At the level of the peritubular capillaries, extensive duplication of the peritubular basement membrane, as seen with electron microscopy, is associated with chronic rejection (423). Furthermore, characteristic lesions in the glomerulus (transplant glomerulopathy), characterized by duplication of the GBM (figure 4) also points to the involvement of chronic rejection. In recent years, the role of chronic humoral rejection (or chronic antibody mediated rejection) as a cause of chronic allograft dysfunction has received renewed attention. During anti-donor HLA antibody mediated rejection, a component of the complement system is found to remain covalently bound to the surface of endothelial cells. This component, C4d, can be visualized by immunohistochemistry, and indicates an ongoing or previous antibody mediated rejection (424,425) (figure 5). In 88 percent of patients that showed widespread C4d positivity in peritubular capillaries circulating anti-donor HLA antibodies were found, compared to none of the C4d negative patients (416), indicating an ongoing humoral rejection.

Calcineurin inhibitor toxicity

Calcineurin inhibitors (CNIs), such as cyclosporine A and tacrolimus are very potent immunosuppressive drugs that are widely used in transplantation. By inhibiting the enzyme calcineurin these compounds suppress IL-2 production and thus prevent maturation of T-cells. Already in the first report on cyclosporine A a nephrotoxic side effect was mentioned (357). In subsequent years, the histopathology of the chronic form of CNI toxicity – although structurally unrelated, cyclosporine A and tacrolimus cause identical histopathologic lesions (426) – has been described, mostly

through studies by Mihatsch and coworkers (427-429). Studies in non-kidney transplantation in which cyclosporine A was used have been instrumental, since the renal effects of cyclosporine can be studied in the absence of other types of damage to the kidney (430).

Chronic CNI toxicity develops after months to years of CNI use, and in large series it is related to the dose. Since the bioavailability is highly variable in individual patients, dosage of CNIs is not a good correlate of CNI exposure. Sophisticated drug-monitoring and dosing may help overcome this problem.

The scope of the problem seems to be considerable. In the Leiden University Medical Center, 20 percent of a cohort of patients that switched from once daily Sandimmune to twice-daily Neoral (two different formulations of cyclosporine A) developed chronic cyclosporine A toxicity, even though they received their medication according to nationwide guidelines (431). In this study, cyclosporine toxicity was defined by functional and histological criteria, ie, a decline in renal function temporally related to the switch in cyclosporine A formulation that could not be explained by other features in the renal biopsy. The number of patients that show histological signs of CNI toxicity after long-term treatment with CNIs may be even higher. In the aforementioned study by Nankivell et al CNI toxicity became 'virtually universal' by 10 years after transplantation (420).

Similar to the chronic lesions seen in CAN, the histopathology of chronic CNI toxicity is characterized by tubular atrophy and interstitial fibrosis, representing a nonspecific response to damage. The fibrosis has been reported to affect rays of cortex and medulla, leading to so-called 'striped fibrosis'. This band-pattern of fibrosis is thought to be caused by a watershed infarction, ischemia in the debit of a larger artery affected by cyclosporine. The lesion is typical but not specific for CNI toxicity (432), and its value is debated.

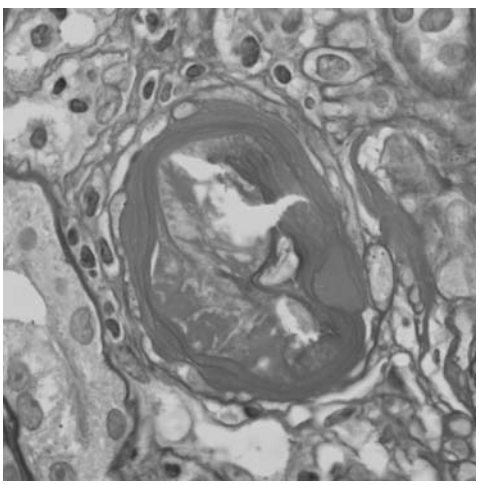


Figure 6. Arteriolar hyalinosis. Nodular depositions of hyaline material are seen in the media of arterioles, indicating the presence of chronic CNI toxicity.

Glomerular lesions are also common in chronic CNI toxicity and include glomerular hypertrophy, mesangial matrix expansion, and focal segmental glomerular sclerosis. Again, these changes are of limited specificity (433). The most specific lesion of chronic CNI toxicity is arteriolar hyalinosis, in which nodular depositions of hyaline material are seen in the media of arterioles (figure 6). Pathogenetically, CNIs are thought to damage arteriolar smooth muscle cells that are subsequently replaced by hyaline material from the circulation (427,433). In advanced cases, the hyalinosis may be circumferential (434), and severely narrow the vascular lumen.

Nevertheless, use of arteriolar hyalinosis as a marker of chronic CNI toxicity has certain drawbacks. For example, lesions that are characteristic for CNI toxicity appear focally in the tissue and thus carry the risk of sampling error (435). Also, the interobserver consistency of scoring renal biopsies has been low (436,437). Attempts have been made to increase the reproducibility of arteriolar hyalinosis scoring by introducing a different classification (434). This new classification system seemed to outperform the previous one, but it is questionable whether this is due to a real improvement or just to a reduction of categories in the new scoring system (438). Another complicating factor in the use of arteriolar hyalinosis as a marker of CNI toxicity is that this lesion is also seen in association with other diseases such as diabetic nephropathy, hypertension, and hemolytic uremic syndrome / thrombotic microangiopathy (439).

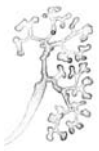
Although chronic rejection and chronic CNI toxicity are both individual causes of chronic allograft dysfunction, they can coexist and contribute to the histopathological picture of CAN.

Pathophysiologic processes in chronic allograft dysfunction

There are several non-mutually exclusive pathophysiologic processes that damage the kidney and lead to development of chronic allograft dysfunction and CAN, often in a way that is similar to the progression of renal disease in native kidneys. Indeed, the kidney responds to injury in a relatively stereotypic manner, eventually culminating in replacement of functional nephrons by sclerosed glomeruli, atrophic tubuli, and interstitial fibrosis. The shared features and final common pathway of renal disease progression is highlighted in a previous paragraph. Those of special interest in the setting of transplantation will be discussed here and include alloimmunity, immunosuppressive drug toxicity, and accelerated senescence.

Alloimmunity

Shishido et al found a relation between subclinical acute rejection and extent of CAN (387). This implies that cellular infiltrates continue to inflict damage on the allograft on the long term. This continuing inflammation will lead to a destruction of the tissue, and may in addition lead to an enhanced allorecognition due to an increase in MHC expression (369), resulting in a self-perpetuating cycle of injury and inflammation. At the same time, not all cellular infiltrates are associated with the development of CAN (421), suggesting that cellular infiltrates differ in their destructive properties.



An interesting process contributing to the immunological cause of chronic allograft nephropathy was recently described in a series of studies by Kerjaschki. Using relatively novel markers for lymphatic endothelial cells, including the glycoprotein podoplanin, they found that the number of lymphatic vessels was increased over 50-fold in allografts that showed nodular infiltrates (150). In a follow-up paper they showed that these newly formed lymphatics are in part derived from recipient lymphatic progenitor cells, possibly macrophages (440,441). The lymphatics were co-localized with a nodular infiltrate that contained active and dividing T and B lymphocytes, macrophages and dendritic cells, resembling an intragraft lymphoid organ. Moreover, the lymphatic endothelial cells were shown to produce cytokines that would enable them to recruit lympocytes and thus initiate or sustain an anti-allograft immune response. Not only could this response be of a cellular nature, this study and several other studies also found evidence for the involvement of B-cells in rejection (442).

Indeed, humoral allograft rejection has received renewed attention in the last decade since the discovery of C4d as a marker of chronic rejection coinciding with the presence of circulating antidonor antibodies (see above). The pathogenic processes will only be described briefly, and have been reviewed in detail by several authors (367,424). Antidonor antibodies are generally directed against donor HLA. Dendritic cells of the donor (the direct pathway) or the recipient (the indirect pathway) present donor HLA antigens to recipient T-cells. This activates the T-cells that, through production of a series of cytokines and co-stimulatory molecules such as CD28 and CD40, activate the B-cell (turning them into plasma cells) and thus initiate formation of antidonor antibodies. These antibodies bind to donor tissue, predominantly endothelial cells, and cause complement fixation, leading to formation of the membrane attack complex, attraction of other inflammatory cells, and activation of endothelial cells. This either leads to apoptosis of endothelial cells, or, if the cells survive, production of inflammation promoting factors such as intracellular adhesion molecule 1 (ICAM), vascular cell adhesion molecule 1 (VCAM) and E-selectin, and other chemotactic cytokines, providing a link to cellular immunity. Also, the cytokines produced by activated endothelial cells may be harmful to the kidney.

Circulating antibodies may also be directed against non-HLA antigens. Joosten et al described the presence of antibodies directed against the GBM proteoglycan agrin in patients with transplant glomerulopathy (443).

Immunosuppressive drug toxicity

Immunosuppressive drugs, in particular CNIs, may promote allograft damage through a variety of processes, including vasoconstriction, direct cellular toxicity, and modulation of fibrosis-related gene transcription. CNIs are known to increase vascular resistance and promote vasoconstriction. This may result from an inhibition of nitric oxide production or an increased release of renin from

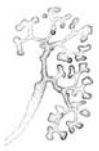
the juxtaglomerular apparatus, activating the renin-angiotensin system (RAS) (414,444). Also, necrosis of arteriolar smooth muscle cells and replacement by hyaline material may contribute to the obliteration of the vascular lumen (432). The resulting impaired blood flow may lead to ischemia, and result in glomerular and tubulointerstitial fibrosis. Also, CNIs may have a direct cytotoxic effect on renal cells, including podocytes (158), but results have been controversial with regard to tubular epithelial cells (444).

Furthermore, CNIs increase the expression of profibrotic and proinflammatory cytokines, as well as interstitial matrix molecules, either directly or via stimulation of the RAS (445). Among the cytokines influenced by CNIs are TGF-β (446-448), osteopontin (448), ET-1, MCP-1, and RANTES (449). Cyclosporine A was shown to stimulate the promoter of the collagen III gene in monkey fibroblast cells (450), and increased the expression of collagen I in human and experimental renal cortical tissue (448,451). Recently, it was described that cyclosporine A may induce epithelial-to-mesenchymal transition (EMT) of tubular epithelial cells, and thus contribute to interstitial fibrosis (452). The mechanism of EMT is discussed in more detail in a previous paragraph.

Another aspect of immunosuppressive medication is that it increases the chance of viral infections of the allograft. For example, cytomegalo virus infection and BK virus nephropathy (BK virus is named after the patient in whom the infection was first described (453)) have been related to the development of chronic allograft dysfunction. This may take place through stimulation of immunological pathways leading to chronic inflammation (365,454) as well as direct damage of renal epithelial cells (455).

Accelerated senescence

As mentioned previously, donor age is an important risk factor for the development of chronic allograft dysfunction. One pathophysiologic concept that has been raised to explain this association is the so-called accelerated senescence of renal allografts (369). In vitro, after a limited number of cell cycles, cells become quiescent and usually die. This 'cellular senescence' is characterized by a number of factors. The length of the telomeres of chromosomes, for example, decreases with each replication, and in senescent cells, cell cycle inhibitory molecules such as p16 and p21 are upregulated. Halloran and co-workers noted that the histopathological characteristics of ageing overlap with that of CAN. They studied the expression of p16, and found that this protein was expressed in increased amounts in allografts with inferior function and with signs of CAN (456). Similarly, Ferlicot et al found that the severity of CAN was correlated with the extent of telomere shortening (457). These data support the hypothesis, as put forward by Halloran (369), that the multiple stresses that the allograft encounters would put replicative stress on the allograft kidney cells, thereby accelerating cellular senescence. Indeed, in an animal model of chronic rejection studied by Joosten et al, stressors such as warm ischemia time correlated with the expression of



p16 and p21 as well as with telomere shortening in the tubular epithelial cells, although this was not exclusively associated with chronic rejection (458). Together, these data suggest that replicative senescence may be a pathophysiologic pathway of progression in both native and allograft kidney diseases: once the continuously stressed cells have reached their replicative limit, they are unable to effectively keep up their normal response to damage, leading to atrophy and fibrosis rather than reepithelialization and ECM remodeling.

Molecular diagnostics in chronic allograft dysfunction

The clinical course of long-term graft dysfunction is aspecific, and often does not provide a firm basis for therapeutic decision making. As pointed out in the previous paragraphs, histopathological evaluation of renal biopsies may help in defining the cause of renal function loss, but also has limitations.

This has urged researchers to search for markers that might complement or even replace routine histopathologic evaluation. Such studies have been performed using hypothesis-driven approaches, in which parts of the pathogenesis of renal transplant pathology were studied. Others have utilized broader approaches, for example through application of microarray and proteomics. A summary of these studies is given in the following paragraphs, with a focus on studies on chronic allograft dysfunction in humans. For a broader scope on the subject, see more thorough reviews of the use of molecular markers in native kidney diseases (459-462), and acute rejection (463,464).

Diagnostic markers at the tissue level

The extent of interstitial fibrosis is the most important histological predictor of progression of renal disease in both native and allograft kidneys. However, semi-quantitative scales as provided by the Banff classification impair the reliable measurement of the extent of interstitial fibrosis in renal allografts (436,437). To circumvent this problem, several researchers have used a semi-automated approach using digital image analysis. Nicholson et al studied the predictive properties of quantitative immunohistochemistry in protocol transplant biopsies. They found that the collagen type III positive area at 6 months was correlated with renal function at 12 and 24 months after transplantation (465). Others have used Sirius Red, a red dye that intercalates with the interstitial collagens I and III, to stain the interstitial compartment (466). These studies showed that computerized measurement of the Sirius Red positive material in biopsies taken early after

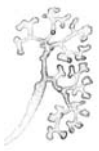
transplantation is correlated with the long term decline in renal function and may outperform the semi-quantitative scoring method (467,468). Quantification of interstitial fibrosis using Sirius Red has also proven to be useful as a surrogate marker of CAN, for example to compare fibrogenic effects of different types of CNIs (418) and long term effects of subclinical acute rejection (421).

One of the reasons to use interstitial fibrosis and tubular atrophy for grading of chronic allograft nephropathy in the Banff criteria is that these changes are fairly common in renal allograft biopsies and therefore less subject to sampling error (422). Studies in animal models of glomerulosclerosis made clear that the composition of glomerular extracellular matrix harbors disease specific features (469). Similarly, it is conceivable that the composition of the interstitial fibrotic lesions varies with the cause of the disease (470). Thus, studying composition of the interstitial fibrosis may provide valuable markers and overcome the sampling error limitations of the abovementioned histopathological markers.

Following this approach, Abrass et al (471) studied the composition of the extracellular matrix of renal allograft biopsies by staining for different interstitial collagens (I, III, and IV), laminins, fibronectin, and thrombospondin. In addition, they studied particular collagen IV and laminin chains. In a pilot study, they identified three different patterns of ECM composition in renal allograft biopsies. Pattern 1 showed no change in comparison to normal interstitium, pattern 2 showed an generalized accumulation of collagens I and III, while pattern 3 showed a de novo expression of collagen α3(IV) and laminin β2 in the proximal tubular basement membrane. Subsequently, they studied the presence of these patterns in biopsies from patients with either chronic rejection or chronic cyclosporine A toxicity. Chronic rejection was exclusively associated with the third pattern, indicating that indeed ECM patterns could help distinguish the two causes of late allograft dysfunction (471).

Other molecular markers at the tissue level have proven to be useful in the diagnosis of specific causes of chronic allograft dysfunction. For example, as discussed above, the complement split product C4d has become integrated in routine diagnostics as a marker of humoral rejection.

In conclusion, studying changes at the tissue level – using conventional histologic as well as immunohistochemical approaches – can help predict the clinical course and may help distinguish different causes of allograft dysfunction. The downside of these techniques is that the damage has to be present, at least to some extent, to make the diagnosis. This probably limits the success of subsequent treatments (472). In this context, analysis of mRNA expression levels could be promising. This will be lined out in the following section.



Genomics

Single gene measurements

In the early 1990s, studies in experimental renal disease showed that the mRNA expression levels of ECM components such as collagen I and IV correlated with the severity of glomerulosclerosis (473,474). Moreover, the changes in mRNA levels preceded the morphological changes (475). These studies raised the concept, as reviewed by Striker (476), that studying mRNA expression levels could be of predictive value with regard to both occurrence and severity of renal disease.

This concept has been elaborately tested in the setting of transplantation. Measurements of mRNA expression levels of immune activation genes in renal allografts proved to be a tool to diagnose the presence of acute rejection (477-479). Furthermore, such measurements can be performed non-invasively using urinary cells. The group of Suthanthiran showed that urinary mRNA expression levels of transcripts related to rejection (granzyme B and perforin) and regulatory T-cells (FOXP3) predict the presence of acute rejection (480) and reversal of acute rejection (481), respectively. Although these results remain to be confirmed in larger patient groups (482), and although studying the urine may neglect valuable information that can exclusively be found in the renal biopsy (483), it clearly shows the strength of molecular analysis.

Acute rejection episodes are associated with profound and quick alterations in the cellular composition of the graft, and it is in this regard not surprising that this is reflected in differences in mRNA expression. Can molecular biological techniques be applied to the more indolent development of chronic allograft dysfunction? Because CAN is characterized by interstitial fibrosis, researches have initially focused on ECM components and profibrogenic cytokines. Sharma et al were the first to describe a relation between elevated mRNA expression levels of the profibrogenic cytokine TGF-β and the presence of CAN (484), a finding later also reported in protocol biopsies (419). Expression levels of TGF-β mRNA in the urinary cells of patient with CAN also proved to be increased (485). Suthanthiran also found that TGF-β mRNA levels were related to 'chronic rejection' and interstitial fibrosis, but found no relation to acute rejection (479). This may be explained by the fact that besides its profibrogenic properties, TGF-β has a strong immunosuppressive effect. Eikmans et al (486) found that an upregulation of TGF-β mRNA in biopsies from patients with early acute rejection was associated with the absence of the development of chronic allograft dysfunction, underscoring the dual action of this cytokine. Scherer et al found similar results using a microarray approach (487). Plasminogen activator inhibitor 1 (PAI-1) is another profibrotic cytokine that has been implicated in the development of interstitial fibrosis. Delarue et al found that increased levels of PAI-1 mRNA correlated with a decline in renal function over 5 years of follow-up (488). Increased PAI-1 expression has also been observed at the protein level in CAN (489).

Not only fibrogenesis, but also an impaired ECM turnover may result in interstitial fibrosis. Indeed increased transcripts of tissue inhibitors of metalloproteinases – proteins that inhibit matrix degrading enzymes – are related to the extent of interstitial fibrosis (490).

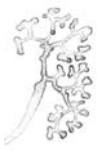
In keeping with the multifactorial etiology of chronic allograft dysfunction, others found that the expression of immunologic transcripts was related to chronic allograft nephropathy (491-493), although this has not been a consistent finding (484).

The problem of prediction

Most of the studies listed so far describe associations between mRNA transcripts and development of chronic allograft dysfunction. Demonstration of various mRNA transcripts in chronically deteriorating kidneys may help unravel the pathogenesis of CAN. Also, comparable to the use of quantitative measurements of interstitial fibrosis as a surrogate marker for CAN, mRNA expression analysis has been instrumental in determining the fibrotic response to different immunosuppressive treatment modalities (494). Still, the ultimate goal of mRNA expression analysis would be to find markers that predict development of CAN. Few studies have actually tested the predictive value of investigated markers (495). In a protocol biopsy study, Baboolal et al found that, although TGF-β, thrombospondin and fibronectin were upregulated in biopsies that showed CAN, none of these markers alone could reliably indicate renal injury, and acute rejection and interstitial fibrosis continued to have the most profound impact on prognosis (419).

Microarray studies

The problem, that many genes are associated with CAN but few predict its development, has become more apparent after a landmark study by Sarwal et al (442). This microarray study on 52 pediatric renal transplant patients, the largest microarray study performed in transplantation so far, has raised a number of intriguing concepts. Clustering of the samples showed considerable heterogeneity in biopsies with acute rejection. One of the acute rejection clusters contained a large number of B-cell markers, again suggesting an important role for humoral rejection (see above) and opening new directions for therapy. However, such a heterogeneity was not found in the samples that were on histological criteria diagnosed as CAN (442). In a review on the use of microarray in transplantation, Sarwal et al state that the late sampling of CAN biopsies impairs the identification of specific causes of CAN. The differential regulation of genes compared to stable allografts at the late stage only reflects the ongoing damage mechanisms (495). This is exemplified by a study by Hotchkiss and co-workers, who studied 16 transplant biopsies with CAN with and without arteriolar hyalinosis in comparison to 6 normal transplant biopsies using microarray (496). They found that the CAN biopsies were clearly different from the controls, but – similar to Sarwals report – did not find differences between CAN subgroups. They did find genes known to be involved in CAN pathogenesis, but concluded that for more insight in CAN development, earlier biopsies should be studied. Donauer et al compared 13 transplants with



chronic transplant failure with normal controls and end stage renal disease (497). They found that the end-stage renal disease kidneys, either transplants or native kidneys, clustered together, suggesting a shared pathway to chronic renal failure. Moreover, the chronically rejected kidneys showed two distinct subsets at the transcriptional level, but these could not be traced back to clinical or histological differences.

In conclusion, microarray studies in the field of chronic allograft dysfunction have shown that it is difficult to obtain information on different pathogenetic pathways or markers of disease progression once CAN has started to develop. Instead, strategies should aim at defining such markers early in the course after transplantation.

A number of microarray studies have used this approach to more specifically address the predictive value of gene expression measurements. Scherer et al (487) defined two groups that showed a divergent course of allograft function between 6 and 12 months after transplantation. Using microarray, they selected and validated a set of 10 genes (eight up- and two downregulated) that could differentiate between the two groups. Eikmans et al defined two groups of patients that both experienced acute rejection but diverged in their long-term follow up. Biopsies of the two groups, designated progressors and non-progressors, were studied using microarray, and a number of differentially regulated genes were further analyzed with respect to their predictive properties in a separate group of patients (498). Surfactant protein C showed the best predictive value of long-term outcome (498), while the combination of several array-selected markers in addition to conventional histological and clinical parameters had an even superior predictive performance (M. Eikmans, personal communication).

Proteomics, metabolomics, urinomics

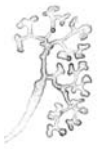
It is imaginable that the large scale-analysis of proteins would provide extra tools for monitoring renal transplant function and identification of complications. The validity of this concept has been scarcely tested in transplantation, but there are some examples of recognition of acute rejection using a proteomic approach with sensitivity and specificity comparable to that of mRNA analysis (499,500). Whether this also holds true for chronic allograft dysfunction remains to be established, but it should be kept in mind that the proteomics approach may meet the same caveats as discussed in relation to genomics. Metabolomics is even more in its infancy (with reviews on the subject outnumbering original research publications) but may hold promise for the development of novel and – if measured in urine – non-invasive biomarkers for transplant monitoring (501).

Outline of the thesis

Two relatively distinct topics are covered in this thesis: the molecular mechanisms related to the development of proteinuria, and those related to kidney transplant failure. Our interest in the development of proteinuria was triggered by the discovery of several genes mutated in hereditary nephrotic syndromes. Following these findings, it was suggested that the genes and respective proteins might likewise be involved in the development of acquired proteinuric diseases. This was the subject of the study described in chapter 2, in which we compared the regulation of several podocyte-associated genes and proteins in relation to podocyte morphology in patients with and without proteinuria. Studying human material often restricts the evaluation of changes in time, which was the reason to use a rat model for follow-up studies on the same subject: the regulation of podocyte molecules during the development of proteinuria. In chapter 3, the results of a time-course study of the spontaneously proteinuric Dahl salt-sensitive rat in comparison to the non-proteinuric spontaneously hypertensive rat are described. This is a largely descriptive study that focuses on the glomerular changes in the early phase of proteinuria development. In an attempt to get more insight in the cause of proteinuria in this rat model, we performed a microarray study using the same rat model, of which the findings are described in chapter 4.

Proteinuria is an important risk factor for the progression of renal disease, both in setting native and transplant diseases. Upon initial injury, kidneys succumb to a progressive course of renal function loss that is histologically characterized by the replacement of functional tissue by scar tissue, a process referred to as interstitial fibrosis. This process seems to be uncoupled from the initial cause of injury, although this injury may still persist in the progressively fibrotic kidney. This makes it difficult to install treatments directed at the etiology of the disease. This is especially true in the transplantation setting, in which different kinds of injury give rise to similar histopathological presentations. We were mainly interested in two distinct causes of kidney transplant injury that both operate in the late phase after transplantation: the toxic effects of the immunosuppressant cyclosporine A, versus the injurious effects of chronic immunologic activity. Although the clinical and histopathological presentation of these two causes is relatively similar, the modes of treatment obviously differ. We tried to dissect the role of the different causes by focusing on the differences in mRNA expression in two well-defined groups of patients suffering from either disease; the results of this study are described in chapter 5. In a similar patient group, we studied the protein composition of the interstitial fibrosis, which is the subject of chapter 6.

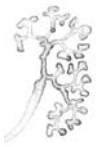
In chapter 7 these studies are summarized and placed in a more general perspective.



References

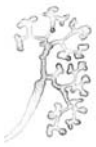
1. Smith HW: From fish to philosopher: the story of our internal environment. New Jersey, Summit, CIBA , 1959
2. Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 112:1776-1784, 2003
3. Bates CM: Kidney development: regulatory molecules crucial to both mice and men. Mol Genet Metab 71:391-396, 2000
4. Heptinstall RH, Emancipator SN, Hill GS, Kriz W, McCluskey RT, Olson JL, Porter KA, Risdon RA, Schwartz MM, Silva FG, Solez K, Venkatachalam MA: Pathology of the Kidney, 4th edn. Boston, Little, Brown and Company, 1992
5. Horster MF, Braun GS, Huber SM: Embryonic renal epithelia: induction, nephrogenesis, and cell differentiation. Physiol Rev 79:1157-1191, 1999
6. Larsen WJ: Human Embryology, 2 edn. New York, Churchill Livingstone, 1997
7. Saxen L: Organogenesis of the Kidney, 1 edn. New York, Cambridge University Press, 1987
8. Matsumoto T, Winkler CA, Brion LP, Schwartz GJ: Expression of acid-base-related proteins in mesonephric kidney of the rabbit. Am J Physiol 267:F987-F997, 1994
9. Rothenpieler UW, Dressler GR: Pax-2 is required for mesenchyme-to-epithelium conversion during kidney development. Development 119:711-720, 1993
10. Torban E, Dziarmaga A, Iglesias D, Chu LL, Vassilieva T, Little M, Eccles M, Discenza M, Pelletier J, Goodyer P: PAX2 activates WNT4 expression during mammalian kidney development. J Biol Chem 281:12705-12712, 2006
11. Stark K, Vainio S, Vassileva G, McMahon AP: Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. Nature 372:679-683, 1994
12. Zeisberg M: Bone morphogenic protein-7 and the kidney: current concepts and open questions. Nephrol Dial Transplant 21:568-573, 2006
13. Ryan G, Steele-Perkins V, Morris JF, Rauscher FJ, III, Dressler GR: Repression of Pax-2 by WT1 during normal kidney development. Development 121:867-875, 1995
14. Pavenstadt H, Kriz W, Kretzler M: Cell biology of the glomerular podocyte. Physiol Rev 83:253-307, 2003
15. Sanden SK, Wiggins JE, Goyal M, Riggs LK, Wiggins RC: Evaluation of a thick and thin section method for estimation of podocyte number, glomerular volume, and glomerular volume per podocyte in rat kidney with Wilms' tumor-1 protein used as a podocyte nuclear marker. J Am Soc Nephrol 14:2484-2493, 2003
16. Gubler MC: Podocyte differentiation and hereditary proteinuria/nephrotic syndromes. J Am Soc Nephrol 14:S22-S26, 2003
17. Kreidberg JA: Podocyte Differentiation and Glomerulogenesis. J Am Soc Nephrol 14:806-814, 2003
18. Simon M, Grone HJ, Johren O, Kullmer J, Plate KH, Risau W, Fuchs E: Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and in adult kidney. Am J Physiol 268:F240-F250, 1995
19. Tufro A, Norwood VF, Carey RM, Gomez RA: Vascular endothelial growth factor induces nephrogenesis and vasculogenesis. J Am Soc Nephrol 10:2125-2134, 1999
20. Saxen L, Sariola H: Early organogenesis of the kidney. Pediatr Nephrol 1:385-392, 1987
21. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest 111:707-716, 2003
22. Eremina V, Cui S, Gerber H, Ferrara N, Haigh J, Nagy A, Ema M, Rossant J, Jothy S, Miner JH, Quaggin SE: Vascular endothelial growth factor a signaling in the podocyte-endothelial compartment is required for mesangial cell migration and survival. J Am Soc Nephrol 17:724-735, 2006
23. Lindahl P, Hellstrom M, Kalen M, Karlsson L, Pekny M, Pekna M, Soriano P, Betsholtz C: Paracrine PDGF-B/PDGF-Rbeta signaling controls mesangial cell development in kidney glomeruli. Development 125:3313-3322, 1998
24. Dressler GR, Woolf AS: Pax2 in development and renal disease. Int J Dev Biol 43:463-468, 1999
25. Rostgaard J, Qvortrup K: Electron Microscopic Demonstrations of Filamentous Molecular Sieve Plugs in Capillary Fenestrae. Microvascular Research 53:1-13, 1997
26. Deen WM: What determines glomerular capillary permeability? J Clin Invest 114:1412-1414, 2004
27. Ballermann BJ, Stan RV: Resolved: Capillary Endothelium Is a Major Contributor to the Glomerular Filtration Barrier. J Am Soc Nephrol 18:2432-2438, 2007
28. Kriz W, Elger M, Mundel P, Lemley KV: Structure-stabilizing forces in the glomerular tuft. J Am Soc Nephrol 5:1731-1739, 1995
29. Neal CR, Crook H, Bell E, Harper SJ, Bates DO: Three-dimensional reconstruction of glomeruli by electron microscopy reveals a distinct restrictive urinary subpodocyte space. J Am Soc Nephrol 16:1223-1235, 2005
30. D'Agati V: And You Thought the Age of Anatomic Discovery Was Over. J Am Soc Nephrol 16:1166-1168, 2005
31. Kriz W, Hackenthal E, Nobiling R, Sakai T, Elger M, Hahnel B: A role for podocytes to counteract capillary wall distension. Kidney Int 45:369-376, 1994

32. Bariety J, Mandet C, Hill GS, Bruneval P: Parietal podocytes in normal human glomeruli. J Am Soc Nephrol 17:2770-2780, 2006
33. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, Elger M, Kriz W, Floege J, Moeller MJ: Recruitment of Podocytes from Glomerular Parietal Epithelial Cells. J Am Soc Nephrol 20:333-343, 2009
34. Smeets B, Dijkman H, Wetzels J, Steenbergen E: Lessons from studies on focal segmental glomerulosclerosis: an important role for parietal epithelial cells? J Pathol 2006
35. Smeets B, Te Loeke NA, Dijkman HB, Steenbergen ML, Lensen JF, Begieneman MP, van Kuppevelt TH, Wetzels JF, Steenbergen EJ: The parietal epithelial cell: a key player in the pathogenesis of focal segmental glomerulosclerosis in Thy-1.1 transgenic mice. J Am Soc Nephrol 15:928-939, 2004
36. Asano T, Niimura F, Pastan I, Fogo AB, Ichikawa I, Matsusaka T: Permanent Genetic Tagging of Podocytes: Fate of Injured Podocytes in a Mouse Model of Glomerular Sclerosis. J Am Soc Nephrol 2005
37. Zhong J, Zuo Y, Ma J, Fogo AB, Jolicoeur P, Ichikawa I, Matsusaka T: Expression of HIV-1 genes in podocytes alone can lead to the full spectrum of HIV-1-associated nephropathy. Kidney Int 68:1048-1060, 2005
38. Dijkman H, Smeets B, van der LJ, Steenbergen E, Wetzels J: The parietal epithelial cell is crucially involved in human idiopathic focal segmental glomerulosclerosis. Kidney Int 68:1562-1572, 2005
39. Dijkman HB, Weening JJ, Smeets B, Verrijp KC, van Kuppevelt TH, Assmann KK, Steenbergen EJ, Wetzels JF: Proliferating cells in HIV and pamidronate-associated collapsing focal segmental glomerulosclerosis are parietal epithelial cells. Kidney Int 70:338-344, 2006
40. Mundel P, Kriz W: Structure and function of podocytes: an update. Anat Embryol (Berl) 192:385-397, 1995
41. Lee LK, Pollock AS, Lovett DH: Asymmetric origins of the mature glomerular basement membrane. J Cell Physiol 157:169-177, 1993
42. Abrahamson DR, John PL, Isom K, Robert B, Miner JH: Partial Rescue of Glomerular Laminin {alpha}5 Mutations by Wild-Type Endothelia Produce Hybrid Glomeruli. J Am Soc Nephrol 18:2285-2293, 2007
43. Haraldsson B, Sorensson J: Why do we not all have proteinuria? An update of our current understanding of the glomerular barrier. News Physiol Sci 19:7-10, 2004
44. Mene P, Simonson MS, Dunn MJ: Physiology of the mesangial cell. Physiol Rev 69:1347-1424, 1989
45. Miner JH: Renal basement membrane components. Kidney Int 56:2016-2024, 1999
46. Ichimura K, Kurihara H, Sakai T: Actin filament organization of foot processes in rat podocytes. J Histochem Cytochem 51:1589-1600, 2003
47. Drenckhahn D, Franke RP: Ultrastructural organization of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat, and man. Lab Invest 59:673-682, 1988
48. Endlich N, Endlich K: Stretch, tension and adhesion - adaptive mechanisms of the actin cytoskeleton in podocytes. Eur J Cell Biol 85:229-234, 2006
49. Somlo S, Mundel P: Getting a foothold in nephrotic syndrome. Nat Genet 24:333-335, 2000
50. Kerjaschki D: Caught flat-footed: podocyte damage and the molecular bases of focal glomerulosclerosis. J Clin Invest 108:1583-1587, 2001
51. Gao SY, Li CY, Shimokawa T, Terashita T, Matsuda S, Yaoita E, Kobayashi N: Rho-family small GTPases are involved in forskolin-induced cell-cell contact formation of renal glomerular podocytes in vitro. Cell Tissue Res 2007
52. Togawa A, Miyoshi J, Ishizaki H, Tanaka M, Takakura A, Nishioka H, Yoshida H, Doi T, Mizoguchi A, Matsuura N, Niho Y, Nishimune Y, Nishikawa S, Takai Y: Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDIalpha. Oncogene 18:5373-5380, 1999
53. Morigi M, Buelli S, Angioletti S, Zanchi C, Longaretti L, Zoja C, Galbusera M, Gastoldi S, Mundel P, Remuzzi G, Benigni A: In Response to Protein Load Podocytes Reorganize Cytoskeleton and Modulate Endothelin-1 Gene: Implication for Permselective Dysfunction of Chronic Nephropathies. Am J Pathol 166:1309-1320, 2005
54. The May-Hegglin and Fechtner Syndrome Consortium: Mutations in MYH9 result in the May-Hegglin anomaly, and Fechtner and Sebastian syndromes. Nat Genet 26:103-105, 2000
55. Smoyer WE, Mundel P, Gupta A, Welsh MJ: Podocyte alpha-actinin induction precedes foot process effacement in experimental nephrotic syndrome. Am J Physiol 273:F150-F157, 1997
56. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodriguez-Perez JC, Allen PG, Beggs AH, Pollak MR: Mutations in ACTN4, encoding a-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet 24:251-256, 2000
57. Yao J, Le TC, Kos CH, Henderson JM, Allen PG, Denker BM, Pollak MR: Alpha-actinin-4-mediated FSGS: an inherited kidney disease caused by an aggregated and rapidly degraded cytoskeletal protein. PLoS Biol 2:e167, 2004
58. Kos CH, Le TC, Sinha S, Henderson JM, Kim SH, Sugimoto H, Kalluri R, Gerszten RE, Pollak MR: Mice deficient in alpha-actinin-4 have severe glomerular disease. J Clin Invest 111:1683-1690, 2003
59. Smoyer WE, Ransom RF: Hsp27 regulates podocyte cytoskeletal changes in an in vitro model of podocyte process retraction. FASEB J 16:315-326, 2002
60. Smoyer WE, Gupta A, Mundel P, Ballew JD, Welsh MJ: Altered expression of glomerular heat shock protein 27 in experimental nephrotic syndrome. J Clin Invest 97:2697-2704, 1996
61. Smoyer WE, Mundel P: Regulation of podocyte structure during the development of nephrotic syndrome. J Mol Med 76:172-183, 1998
62. Mundel P, Gilbert P, Kriz W: Podocytes in glomerulus of rat kidney express a characteristic 44 KD protein. J Histochem Cytochem 39:1047-1056, 1991



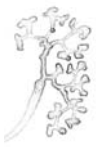
63. Mundel P, Heid HW, Mundel TM, Kruger M, Reiser J, Kriz W: Synaptopodin: an actin-associated protein in telencephalic dendrites and renal podocytes. J Cell Biol 139:193-204, 1997
64. Asanuma K, Kim K, Oh J, Giardino L, Chabanis S, Faul C, Reiser J, Mundel P: Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner. J Clin Invest 115:1188-1198, 2005
65. Huber TB, Kwoh C, Wu H, Asanuma K, Godel M, Hartleben B, Blumer KJ, Miner JH, Mundel P, Shaw AS: Bigenic mouse models of focal segmental glomerulosclerosis involving pairwise interaction of CD2AP, Fyn, and synaptopodin. J Clin Invest 116:1337-1345, 2006
66. Asanuma K, Yanagida-Asanuma E, Faul C, Tomino Y, Kim K, Mundel P: Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling. Nat Cell Biol 8:485-491, 2006
67. Mohos SC, Skoza L: Glomerular sialoprotein. Science 164:1519-1521, 1969
68. Kerjaschki D, Sharkey DJ, Farquhar MG: Identification and characterization of podocalyxin--the major sialoprotein of the renal glomerular epithelial cell. J Cell Biol 98:1591-1596, 1984
69. Orlando RA, Takeda T, Zak B, Schmieder S, Benoit VM, McQuistan T, Furthmayr H, Farquhar MG: The Glomerular Epithelial Cell Anti-Adhesin Podocalyxin Associates with the Actin Cytoskeleton through Interactions with Ezrin. J Am Soc Nephrol 12:1589-1598, 2001
70. Takeda T, Go WY, Orlando RA, Farquhar MG: Expression of podocalyxin inhibits cell-cell adhesion and modifies junctional properties in Madin-Darby canine kidney cells. Mol Biol Cell 11:3219-3232, 2000
71. Doyonnas R, Kershaw DB, Duhme C, Merkens H, Chelliah S, Graf T, McNagny KM: Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. J Exp Med 194:13-27, 2001
72. Takeda T, McQuistan T, Orlando RA, Farquhar MG: Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton. J Clin Invest 108:289-301, 2001
73. Schmieder S, Nagai M, Orlando RA, Takeda T, Farquhar MG: Podocalyxin activates RhoA and induces actin reorganization through NHERF1 and Ezrin in MDCK cells. J Am Soc Nephrol 15:2289-2298, 2004
74. Vogtlander NP, Dijkman H, Bakker MA, Campbell KP, van d, V, Berden JH: Localization of alpha-dystroglycan on the podocyte: from top to toe. J Histochem Cytochem 53:1345-1353, 2005
75. Vogtlander NPJ, Tamboer WPM, Bakker MAH, Campbell KP, van der Vlag J, Berden JHM: Reactive oxygen species deglycosilate glomerular [alpha]-dystroglycan. Kidney Int 69:1526-1534, 2006
76. Breiteneder-Geleff S, Matsui K, Soleiman A, Meraner P, Poczewski H, Kalt R, Schaffner G, Kerjaschki D: Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. Am J Pathol 151:1141-1152, 1997
77. Matsui K, Breitender-Geleff S, Soleiman A, Kowalski H, Kerjaschki D: Podoplanin, a novel 43-kDa membrane protein, controls the shape of podocytes. Nephrol Dial Transplant 14 Suppl 1:9-11, 1999
78. Matsui K, Breiteneder-Geleff S, Kerjaschki D: Epitope-specific antibodies to the 43-kD glomerular membrane protein podoplanin cause proteinuria and rapid flattening of podocytes. J Am Soc Nephrol 9:2013-2026, 1998
79. Martin-Villar E, Megias D, Castel S, Yurrita MM, Vilaro S, Quintanilla M: Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. Journal of cell science 119:4541-4553, 2006
80. Wicki A, Lehembre F, Wick N, Hantusch B, Kerjaschki D, Christofori G: Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell 9:261-272, 2006
81. Wharram BL, Goyal M, Gillespie PJ, Wiggins JE, Kershaw DB, Holzman LB, Dysko RC, Saunders TL, Samuelson LC, Wiggins RC: Altered podocyte structure in GLEPP1 (Ptpro)-deficient mice associated with hypertension and low glomerular filtration rate. J Clin Invest 106:1281-1290, 2000
82. Farquhar MG, Wissig SL, Palade GE: Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. J Exp Med 113:47-66, 1961
83. Rodewald R, Karnovsky MJ: Porous substructure of the glomerular slit diaphragm in the rat and mouse. J Cell Biol 60:423-433, 1974
84. 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
85. 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
86. 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
87. Kawachi H, Kurihara H, Topham PS, Brown D, Shia MA, Orikasa M, Shimizu F, Salant DJ: Slit diaphragm-reactive nephritogenic MAb 5-1-6 alters expression of ZO-1 in rat podocytes. Am J Physiol 273:F984-F993, 1997
88. 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
89. Hallman N, Hjelt L, Ahvenainen EK: Nephrotic syndrome in newborn and young infants. Ann Paediatr Fenn 2:227-241, 1956
90. Ruotsalainen V, Ljungberg P, Wartiovaara J, Lenkkeri U, Kestila M, Jalanko H, Holmberg C, Tryggvason K: Nephrin is specifically located at the slit diaphragm of glomerular podocytes. Proc Natl Acad Sci U S A 96:7962-7967, 1999
91. Holthofer H, Ahola H, Solin ML, Wang S, Palmen T, Luimula P, Miettinen A, Kerjaschki D: Nephrin localizes at the podocyte filtration slit area and is characteristically spliced in the human kidney. Am J Pathol 155:1681-1687, 1999
92. Holzman LB, St John PL, Kovari IA, Verma R, Holthofer H, Abrahamson DR: Nephrin localizes to the slit pore of the glomerular epithelial cell. Kidney Int 56:1481-1491, 1999

93. 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
94. Khoshnoodi J, Sigmundsson K, Ofverstedt LG, Skoglund U, Obrink B, Wartiovaara J, Tryggvason K: Nephrin promotes cell-cell adhesion through homophilic interactions. Am J Pathol 163:2337-2346, 2003
95. Tryggvason K: Unraveling the mechanisms of glomerular ultrafiltration: nephrin, a key component of the slit diaphragm. J Am Soc Nephrol 10:2440-2445, 1999
96. Wartiovaara J, Ofverstedt LG, Khoshnoodi J, Zhang J, Makela E, Sandin S, Ruotsalainen V, Cheng RH, Jalanko H, Skoglund U, Tryggvason K: Nephrin strands contribute to a porous slit diaphragm scaffold as revealed by electron tomography. J Clin Invest 114:1475-1483, 2004
97. Putaala H, Soininen R, Kilpelainen P, Wartiovaara J, Tryggvason K: The murine nephrin gene is specifically expressed in kidney, brain and pancreas: inactivation of the gene leads to massive proteinuria and neonatal death. Hum Mol Genet 10:1-8, 2001
98. 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 2002
99. 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
100. Donoviel DB, Freed DD, Vogel H, Potter DG, Hawkins E, Barrish JP, Mathur BN, Turner CA, Geske R, Montgomery CA, Starbuck M, Brandt M, Gupta A, Ramirez-Solis R, Zambrowicz BP, Powell DR: Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to NEPHRIN. Mol Cell Biol 21:4829-4836, 2001
101. Barletta GM, Kovari IA, Verma RK, Kerjaschki D, Holzman LB: Nephrin and Neph1 co-localize at the podocyte foot process intercellular junction and form cis hetero-oligomers ^. J Biol Chem 2003
102. Gerke P, Huber TB, Sellin L, Benzing T, Walz G: Homodimerization and Heterodimerization of the Glomerular Podocyte Proteins Nephrin and NEPH1. J Am Soc Nephrol 14:918-926, 2003
103. Liu G, Kaw B, Kurfis J, Rahmanuddin S, Kanwar YS, Chugh SS: Neph1 and nephrin interaction in the slit diaphragm is an important determinant of glomerular permeability. J Clin Invest 112:209-221, 2003
104. Ihalmo P, Palmen T, Ahola H, Valtonen E, Holthofer H: Filtrin is a novel member of nephrin-like proteins. Biochem Biophys Res Commun 300:364-370, 2003
105. Ihalmo P, Schmid H, Rastaldi MP, Mattinzoli D, Langham RG, Luimula P, Kilpikari R, Lassila M, Gilbert RE, Kerjaschki D, Kretzler M, Holthofer H: Expression of filtrin in human glomerular diseases. Nephrol Dial Transplant 22:1903-1909, 2007
106. Inoue T, Yaoita E, Kurihara H, Shimizu F, Sakai T, Kobayashi T, Ohshiro K, Kawachi H, Okada H, Suzuki H, Kihara I, Yamamoto T: FAT is a component of glomerular slit diaphragms. Kidney Int 59:1003-1012, 2001
107. Ciani L, Patel A, Allen ND, ffrench-Constant C: Mice lacking the giant protocadherin mFAT1 exhibit renal slit junction abnormalities and a partially penetrant cyclopia and anophthalmia phenotype. Mol Cell Biol 23:3575-3582, 2003
108. Roselli S, Gribouval O, Boute N, Sich M, Benessy F, Attie T, Gubler MC, Antignac C: Podocin localizes in the kidney to the slit diaphragm area. Am J Pathol 160:131-139, 2002
109. Simons M, Schwarz K, Kriz W, Miettinen A, Reiser J, Mundel P, Holthofer H: Involvement of lipid rafts in nephrin phosphorylation and organization of the glomerular slit diaphragm. Am J Pathol 159:1069-1077, 2001
110. Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, Shaw AS, Holzman LB, Mundel P: Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. J Clin Invest 108:1621-1629, 2001
111. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler MC, Niaudet P, Antignac C: NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet 24:349-354, 2000
112. Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL, Pericak-Vance MA, Howell DN, Vance JM, Rosenberg PB: A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. Science 308:1801-1804, 2005
113. Reiser J, Polu KR, Moller CC, Kenlan P, Altintas MM, Wei C, Faul C, Herbert S, Villegas I, Avila-Casado C, McGee M, Sugimoto H, Brown D, Kalluri R, Mundel P, Smith PL, Clapham DE, Pollak MR: TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. Nat Genet 2005
114. Huber TB, Schermer B, Muller RU, Hohne M, Bartram M, Calixto A, Hagmann H, Reinhardt C, Koos F, Kunzelmann K, Shirokova E, Krautwurst D, Harteneck C, Simons M, Pavenstadt H, Kerjaschki D, Thiele C, Walz G, Chalfie M, Benzing T: Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. Proc Natl Acad Sci U S A 103:17079-17086, 2006
115. Moeller MJ, Soofi A, Braun GS, Li X, Watzl C, Kriz W, Holzman LB: Protocadherin FAT1 binds Ena/VASP proteins and is necessary for actin dynamics and cell polarization. EMBO J 23:3769-3779, 2004
116. Shih NY, Li J, Cotran R, Mundel P, Miner JH, Shaw AS: CD2AP Localizes to the Slit Diaphragm and Binds to Nephrin via a Novel C-Terminal Domain. Am J Pathol 159:2303-2308, 2001
117. Lehtonen S, Zhao F, Lehtonen E: CD2-associated protein directly interacts with the actin cytoskeleton. Am J Physiol Renal Physiol 283:F734-F743, 2002
118. Shih NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O, Miner JH, Shaw AS: Congenital nephrotic syndrome in mice lacking CD2-associated protein. Science 286:312-315, 1999
119. Lehtonen S, Ryan JJ, Kudlicka K, Iino N, Zhou H, Farquhar MG: Cell junction-associated proteins IQGAP1, MAGI-2, CASK, spectrins, and {alpha}-actinin are components of the nephrin multiprotein complex. PNAS 102:9814-9819, 2005



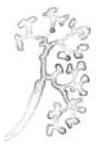
120. Benzing T: Signaling at the slit diaphragm. J Am Soc Nephrol 15:1382-1391, 2004
121. Verma R, Wharram B, Kovari I, Kunkel R, Nihalani D, Wary KK, Wiggins RC, Killen P, Holzman LB: Fyn binds to and phosphorylates the kidney slit diaphragm component Nephrin. J Biol Chem 278:20716-20723, 2003
122. Huber TB, Kottgen M, Schilling B, Walz G, Benzing T: Interaction with podocin facilitates nephrin signaling. J Biol Chem 276:41543-41546, 2001
123. Huber TB, Benzing T: The slit diaphragm: a signaling platform to regulate podocyte function. Curr Opin Nephrol Hypertens 14:211-216, 2005
124. Schiffer M, Bitzer M, Roberts IS, Kopp JB, ten Dijke P, Mundel P, Bottinger EP: Apoptosis in podocytes induced by TGF-b and Smad7. J Clin Invest 108:807-816, 2001
125. Schiffer M, Mundel P, Shaw AS, Bottinger EP: A novel role for the adaptor molecule CD2-associated protein in transforming growth factor-beta-induced apoptosis. J Biol Chem 279:37004-37012, 2004
126. Baelde HJ, Eikmans M, Lappin DWP, Doran PP, Hohenadel D, Brinkkoetter PT, van der Woude FJ, Waldherr R, Rabelink TJ, De Heer E, Bruijn JA: Reduction of VEGF-A and CTGF expression in diabetic nephropathy is associated with podocyte loss. Kidney Int 2007
127. Jones N, Blasutig IM, Eremina V, Ruston JM, Bladt F, Li H, Huang H, Larose L, Li SSC, Takano T, Quaggin SE, Pawson T: Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. Nature 440:818-823, 2006
128. Verma R, Kovari I, Soofi A, Nihalani D, Patrie K, Holzman LB: Nephrin ectodomain engagement results in Src kinase activation, nephrin phosphorylation, Nck recruitment, and actin polymerization. J Clin Invest 116:1346-1359, 2006
129. Tryggvason K, Pikkarainen T, Patrakka J: Nck links nephrin to actin in kidney podocytes. Cell 125:221-224, 2006
130. Moeller MJ: Dynamics at the slit diaphragm--is nephrin actin'? Nephrol Dial Transplant 22:37-39, 2007
131. Kretzler M: Regulation of adhesive interaction between podocytes and glomerular basement membrane. Microsc Res Tech 57:247-253, 2002
132. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. Development 122:3537-3547, 1996
133. Haltia A, Solin M, Luimula P, Kretzler M, Holthofer H: mRNA differential display analysis of nephrotic kidney glomeruli. Exp Nephrol 7:52-58, 1999
134. Guo L, Sanders PW, Woods A, Wu C: The Distribution and Regulation of Integrin-Linked Kinase in Normal and Diabetic Kidneys. Am J Pathol 159:1735-1742, 2001
135. Kretzler M, Teixeira VP, Unschuld PG, Cohen CD, Wanke R, Edenhofer I, Mundel P, Schlondorff D, Holthofer H: Integrin-linked kinase as a candidate downstream effector in proteinuria. FASEB J 15:1843-1845, 2001
136. Blattner SM, Kretzler M: Integrin-linked kinase in renal disease: connecting cell-matrix interaction to the cytoskeleton. Curr Opin Nephrol Hypertens 14:404-410, 2005
137. de Paulo Castro Teixeira V, Blattner SM, Li M, Anders HJ, Cohen CD, Edenhofer I, Calvaresi N, Merkle M, Rastaldi MP, Kretzler M: Functional consequences of integrin-linked kinase activation in podocyte damage. Kidney Int 67:514-523, 2005
138. Yang Y, Guo L, Blattner SM, Mundel P, Kretzler M, Wu C: Formation and Phosphorylation of the PINCH-1-Integrin Linked Kinase-{alpha}-Parvin Complex Are Important for Regulation of Renal Glomerular Podocyte Adhesion, Architecture, and Survival. J Am Soc Nephrol 2005
139. El Aouni C, Herbach N, Blattner SM, Henger A, Rastaldi MP, Jarad G, Miner JH, Moeller MJ, St Arnaud R, Dedhar S, Holzman LB, Wanke R, Kretzler M: Podocyte-Specific Deletion of Integrin-Linked Kinase Results in Severe Glomerular Basement Membrane Alterations and Progressive Glomerulosclerosis. J Am Soc Nephrol 17:1334-1344, 2006
140. Dai C, Stolz DB, Bastacky SI, St.Arnaud R, Wu C, Dedhar S, Liu Y: Essential Role of Integrin-Linked Kinase in Podocyte Biology: Bridging the Integrin and Slit Diaphragm Signaling. J Am Soc Nephrol 17:2164-2175, 2006
141. Raats CJ, van den BJ, Bakker MA, Oppers-Walgreen B, Pisa BJ, Dijkman HB, Assmann KJ, Berden JH: Expression of agrin, dystroglycan, and utrophin in normal renal tissue and in experimental glomerulopathies. Am J Pathol 156:1749-1765, 2000
142. Regele HM, Fillipovic E, Langer B, Poczewki H, Kraxberger I, Bittner RE, Kerjaschki D: Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. J Am Soc Nephrol 11:403-412, 2000
143. 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
144. 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
145. Kerjaschki D, Farquhar MG: Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. J Exp Med 157:667-686, 1983
146. Kerjaschki D: Pathomechanisms and molecular basis of membranous glomerulopathy. Lancet 364:1194-1196, 2004
147. Debiec H, Guigonis V, Mougenot B, Decobert F, Haymann JP, Bensman A, Deschenes G, Ronco PM: Antenatal Membranous Glomerulonephritis Due to Anti-Neutral Endopeptidase Antibodies. N Engl J Med 346:2053-2060, 2002
148. Debiec H, Nauta J, Coulet F, van der Burg M, Guigonisy V, Schurmans T, de Heer E, Soubrier F, Janssen F, Ronco P: Role of truncating mutations in MME gene in fetomaternal alloimmunisation and antenatal glomerulopathies. The Lancet 364:1252-1259, 2004
149. Patrie KM, Drescher AJ, Goyal M, Wiggins RC, Margolis B: The membrane-associated guanylate kinase protein MAGI-1 binds megalin and is present in glomerular podocytes. J Am Soc Nephrol 12:667-677, 2001

150. Kerjaschki D, Regele HM, Moosberger I, Nagy-Bojarski K, Watschinger B, Soleiman A, Birner P, Krieger S, Hovorka A, Silberhumer G, Laakkonen P, Petrova T, Langer B, Raab I: Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. J Am Soc Nephrol 15:603-612, 2004
151. Banas B, Wornle M, Merkle M, Gonzalez-Rubio M, Schmid H, Kretzler M, Pietrzyk MC, Fink M, de Lema GP, Schlondorff D: Binding of the chemokine SLC//CCL21 to its receptor CCR7 increases adhesive properties of human mesangial cells. Kidney Int 66:2256-2263, 2004
152. Kriz W: Biologie des Podozyten. Der Nephrologe V1:144-152, 2006
153. Lahdenkari AT, Lounatmaa K, Patrakka J, Holmberg C, Wartiovaara J, Kestila M, Koskimies O, Jalanko H: Podocytes are firmly attached to glomerular basement membrane in kidneys with heavy proteinuria. J Am Soc Nephrol 15:2611-2618, 2004
154. Eyre J, Ioannou K, Grubb BD, Saleem MA, Mathieson PW, Brunskill NJ, Christensen EI, Topham PS: Statin-sensitive endocytosis of albumin by glomerular podocytes. Am J Physiol Renal Physiol 292:F674-F681, 2007
155. Akilesh S, Huber TB, Wu H, Wang G, Hartleben Br, Kopp JB, Miner JH, Roopenian DC, Unanue ER, Shaw AS: Podocytes use FcRn to clear IgG from the glomerular basement membrane. PNAS 105:967-972, 2008
156. Reddy GR, Pushpanathan MJ, Ransom RF, Holzman LB, Brosius FC, III, Diakonova M, Mathieson P, Saleem MA, List EO, Kopchick JJ, Frank SJ, Menon RK: Identification of the glomerular podocyte as a target for growth hormone action. Endocrinologyen, 2007
157. Xing CY, Saleem MA, Coward RJ, Ni L, Witherden IR, Mathieson PW: Direct effects of dexamethasone on human podocytes. Kidney Int 70:1038-1045, 2006
158. Fornoni A, Li H, Foschi A, Striker GE, Striker LJ: Hepatocyte Growth Factor, but Not Insulin-Like Growth Factor I, Protects Podocytes against Cyclosporin A-Induced Apoptosis. Am J Pathol 158:275-280, 2001
159. Liebau MC, Lang D, Bohm J, Endlich N, Bek MJ, Witherden I, Mathieson PW, Saleem MA, Pavenstadt H, Fischer KG: Functional expression of the renin-angiotensin system in human podocytes. Am J Physiol Renal Physiol 290:F710-F719, 2006
160. Davis BJ, Cao Z, De Gasparo M, Kawachi H, Cooper ME, Allen TJ: Disparate effects of angiotensin II antagonists and calcium channel blockers on albuminuria in experimental diabetes and hypertension: potential role of nephrin. J Hypertens 21:209-216, 2003
161. 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
162. Nagase M, Shibata S, Yoshida S, Nagase T, Gotoda T, Fujita T: Podocyte Injury Underlies the Glomerulopathy of Dahl Salt-Hypertensive Rats and Is Reversed by Aldosterone Blocker. Hypertension 47:1084-1093, 2006
163. Benigni A, Tomasoni S, Gagliardini E, Zoja C, Grunkemeyer JA, Kalluri R, Remuzzi G: Blocking angiotensin II synthesis/activity preserves glomerular nephrin in rats with severe nephrosis. J Am Soc Nephrol 12:941-948, 2001
164. Hoffmann S, Podlich D, Hahnel B, Kriz W, Gretz N: Angiotensin II type 1 receptor overexpression in podocytes induces glomerulosclerosis in transgenic rats. J Am Soc Nephrol 15:1475-1487, 2004
165. Rastaldi MP, Armelloni S, Berra S, Li M, Pesaresi M, Poczewski H, Langer B, Kerjaschki D, Henger A, Blattner SM, Kretzler M, Wanke R, D'Amico G: Glomerular podocytes possess the synaptic vesicle molecule Rab3A and its specific effector rabphilin-3a. Am J Pathol 163:889-899, 2003
166. Kobayashi N, Gao SY, Chen J, Saito K, Miyawaki K, Li CY, Pan L, Saito S, Terashita T, Matsuda S: Process formation of the renal glomerular podocyte: is there common molecular machinery for processes of podocytes and neurons? Anat Sci Int 79:1-10, 2004
167. Cohen CD, Doran PP, Blattner SM, Merkle M, Wang GQ, Schmid H, Mathieson PW, Saleem MA, Henger A, Rastaldi MP, Kretzler M: Sam68-Like Mammalian Protein 2, Identified by Digital Differential Display as Expressed by Podocytes, Is Induced in Proteinuria and Involved in Splice Site Selection of Vascular Endothelial Growth Factor. J Am Soc Nephrol 16:1958-1965, 2005
168. Rastaldi MP, Armelloni S, Berra S, Calvaresi N, Corbelli A, Giardino LA, Li M, Wang GQ, Fornasieri A, Villa A, Heikkila E, Soliymani R, Boucherot A, Cohen CD, Kretzler M, Nitsche A, Ripamonti M, Malgaroli A, Pesaresi M, Forloni GL, Schlondorff D, Holthofer H, D'Amico G: Glomerular podocytes contain neuron-like functional synaptic vesicles. FASEB J 20:976-978, 2006
169. Petermann AT, Pippin J, Hiromura K, Monkawa T, Durvasula R, Couser WG, Kopp J, Shankland SJ: Mitotic cell cycle proteins increase in podocytes despite lack of proliferation. Kidney Int 63:113-122, 2003
170. Kim YG, Alpers CE, Brugarolas J, Johnson RJ, Couser WG, Shankland SJ: The cyclin kinase inhibitor p21CIP1/WAF1 limits glomerular epithelial cell proliferation in experimental glomerulonephritis. 55:2349-2361, 1999
171. Shankland SJ: The podocyte's response to injury: Role in proteinuria and glomerulosclerosis. Kidney Int 70:2131-2147, 2006
172. Moeller MJ, Soofi A, Hartmann I, Le Hir M, Wiggins R, Kriz W, Holzman LB: Podocytes populate cellular crescents in a murine model of inflammatory glomerulonephritis. J Am Soc Nephrol 15:61-67, 2004
173. Ross MJ, Klotman PE: HIV-associated nephropathy. AIDS 18:1089-1099, 2004
174. Barisoni L, Kriz W, Mundel P, D'Agati V: The dysregulated podocyte phenotype: a novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. J Am Soc Nephrol 10:51-61, 1999
175. Albaqumi M, Soos TJ, Barisoni L, Nelson PJ: Collapsing glomerulopathy. J Am Soc Nephrol 17:2854-2863, 2006



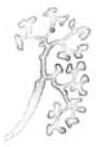
176. Quaggin SE: Transcriptional regulation of podocyte specification and differentiation. Microsc Res Tech 57:208-211, 2002
177. Palmer RE, Kotsianti A, Cadman B, Boyd T, Gerald W, Haber DA: WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. Curr Biol 11:1805-1809, 2001
178. Guo JK, Menke AL, Gubler MC, Clarke AR, Harrison D, Hammes A, Hastie ND, Schedl A: WT1 is a key regulator of podocyte function: reduced expression levels cause crescentic glomerulonephritis and mesangial sclerosis. Hum Mol Genet 11:651-659, 2002
179. Guo G, Morrison DJ, Licht JD, Quaggin SE: WT1 Activates a Glomerular-Specific Enhancer Identified from the Human Nephrin Gene. J Am Soc Nephrol 15:2851-2856, 2004
180. Wagner N, Wagner KD, Xing Y, Scholz H, Schedl A: The Major Podocyte Protein Nephrin Is Transcriptionally Activated by the Wilms' Tumor Suppressor WT1. J Am Soc Nephrol 15:3044-3051, 2004
181. Miner JH, Morello R, Andrews KL, Li C, Antignac C, Shaw AS, Lee B: Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. J Clin Invest 109:1065-1072, 2002
182. Rohr C, Prestel J, Heidet L, Hosser H, Kriz W, Johnson RL, Antignac C, Witzgall R: The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes. J Clin Invest 109:1073-1082, 2002
183. Wearn JT, Richards AN: Observations on the composition of glomerular urine, with particular reference to the problem of reabsorption in the renal tubules. Am J Physiol 71:209-227, 1924
184. Sands JM: Micropuncture: unlocking the secrets of renal function. Am J Physiol Renal Physiol 287:F866-F867, 2004
185. Timio M, Saronio P, Capodicasa E, Timio F: Theories and controversies on urine formation. J Nephrol 16:961-964, 2003
186. Tojo A, Endou H: Intrarenal handling of proteins in rats using fractional micropuncture technique. Am J Physiol Renal Physiol 263:F601-F606, 1992
187. Norden AG, Lapsley M, Lee PJ, Pusey CD, Scheinman SJ, Tam FW, Thakker RV, Unwin RJ, Wrong O: Glomerular protein sieving and implications for renal failure in Fanconi syndrome. Kidney Int 60:1885-1892, 2001
188. 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 288:F605-F613, 2005
189. D'Amico G, Bazzi C: Pathophysiology of proteinuria. Kidney Int 63:809-825, 2003
190. Ohlson M, Sorensson J, Haraldsson B: Glomerular size and charge selectivity in the rat as revealed by FITC-Ficoll and albumin. Am J Physiol Renal Physiol 279:F84-F91, 2000
191. Ohlson M, Sorensson J, Haraldsson B: A gel-membrane model of glomerular charge and size selectivity in series. Am J Physiol Renal Physiol 280:F396-F405, 2001
192. Guasch A, Deen WM, Myers BD: Charge selectivity of the glomerular filtration barrier in healthy and nephrotic humans. J Clin Invest 92:2274-2282, 1993
193. Rennke HG, Cotran RS, Venkatachalam MA: Role of molecular charge in glomerular permeability. Tracer studies with cationized ferritins. J Cell Biol 67:638-646, 1975
194. Brenner BM, Hostetter TH, Humes HD: Glomerular permselectivity: barrier function based on discrimination of molecular size and charge. Am J Physiol Renal Physiol 234:F455-F460, 1978
195. Jeansson M, Haraldsson B: Glomerular Size and Charge Selectivity in the Mouse after Exposure to Glucosaminoglycan-Degrading Enzymes. J Am Soc Nephrol 14:1756-1765, 2003
196. Asgeirsson D, Venturoli D, Rippe B, Rippe C: Increased glomerular permeability to negatively charged Ficoll relative to neutral Ficoll in rats. Am J Physiol Renal Physiol 291:F1083-F1089, 2006
197. Harvey SJ, Miner JH: Breaking Down the Barrier: Evidence against a Role for Heparan Sulfate in Glomerular Permselectivity. J Am Soc NephrolASN, 2007
198. Wijnhoven TJM, Lensen JFM, Wismans RGP, Lamrani M, Monnens LAH, Wevers RA, Rops ALWM, van der Vlag J, Berden JHM, van den Heuvel LPWJ, van Kuppevelt TH: In Vivo Degradation of Heparan Sulfates in the Glomerular Basement Membrane Does Not Result in Proteinuria. J Am Soc NephrolASN, 2007
199. Harvey SJ, Jarad G, Cunningham J, Rops AL, van der Vlag J, Berden JH, Moeller MJ, Holzman LB, Burgess RW, Miner JH: Disruption of Glomerular Basement Membrane Charge through Podocyte-Specific Mutation of Agrin Does Not Alter Glomerular Permselectivity. Am J Patholajpath, 2007
200. Rossi M, Morita H, Sormunen R, Airenne S, Kreivi M, Wang L, Fukai N, Olsen BR, Tryggvason K, Soininen R: Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. EMBO J 22:236-245, 2003
201. Chen S, Wassenhove-McCarthy DJ, Yamaguchi Y, Holzman LB, van Kuppevelt TH, Jenniskens GJ, Wijnhoven TJ, Woods AC, McCarthy KJ: Loss of heparan sulfate glycosaminoglycan assembly in podocytes does not lead to proteinuria. Kidney Int 74:289-299, 2008
202. Rippe B: What is the role of albumin in proteinuric glomerulopathies? Nephrol Dial Transplant 19:1-5, 2004
203. Ryan GB, Karnovsky MJ: Distribution of endogenous albumin in the rat glomerulus: role of hemodynamic factors in glomerular barrier function. Kidney Int 9:36-45, 1976
204. Farquhar MG: Editorial: The primary glomerular filtration barrier--basement membrane or epithelial slits? Kidney Int 8:197-211, 1975
205. Osicka TM, Strong KJ, Nikolic-Paterson DJ, Atkins RC, Comper WD: Albumin and glomerular permselectivity. Nephrol Dial Transplant 19:511-512, 2004
206. Ogston AG, Preston BN: The Exclusion of Protein by Hyaluronic Acid. MEASUREMENT BY LIGHT SCATTERING. J Biol Chem 241:17-19, 1966
207. Deen WM, Lazzara MJ, Myers BD: Structural determinants of glomerular permeability. Am J Physiol Renal Physiol 281:F579-F596, 2001

208. Deen WM: Cellular contributions to glomerular size-selectivity. Kidney Int 69:1295-1297, 2006
209. Tryggvason K, Wartiovaara J: Molecular basis of glomerular permselectivity. Curr Opin Nephrol Hypertens 10:543-549, 2001
210. Tryggvason K: Nephrin: role in normal kidney and in disease. Adv Nephrol Necker Hosp 31:221-234, 2001
211. Tryggvason K, Wartiovaara J: How does the kidney filter plasma? Physiology (Bethesda) 20:96-101, 2005
212. Smithies O: Why the kidney glomerulus does not clog: a gel permeation/diffusion hypothesis of renal function. Proc Natl Acad Sci U S A 100:4108-4113, 2003
213. Rostgaard J, Qvortrup K: Sieve plugs in fenestrae of glomerular capillaries--site of the filtration barrier? Cells Tissues Organs 170:132-138, 2002
214. Bjornson A, Moses J, Ingemansson A, Haraldsson B, Sorensson J: Primary human glomerular endothelial cells produce proteoglycans, and puromycin affects their posttranslational modification. Am J Physiol Renal Physiol 288:F748-F756, 2005
215. Farquhar MG: The glomerular basement membrane: not gone, just forgotten. J Clin Invest 116:2090-2093, 2006
216. Russo PA, Bendayan M: Distribution of endogenous albumin in the glomerular wall of proteinuric patients. Am J Pathol 137:1481-1490, 1990
217. Rippe C, Asgeirsson D, Venturoli D, Rippe A, Rippe B: Effects of glomerular filtration rate on Ficoll sieving coefficients (θ) in rats. Kidney Int 69:1326-1332, 2006
218. Bohman SO, Jaremko G, Bohlin AB, Berg U: Foot process fusion and glomerular filtration rate in minimal change nephrotic syndrome. Kidney Int 25:696-700, 1984
219. Jarad G, Cunningham J, Shaw AS, Miner JH: Proteinuria precedes podocyte abnormalities inLamb2 mice, implicating the glomerular basement membrane as an albumin barrier. J Clin Invest 116:2272-2279, 2006
220. Branten AJ, van den BJ, Jansen JL, Assmann KJ, Wetzels JF: Familial nephropathy differing from minimal change nephropathy and focal glomerulosclerosis. Kidney Int 59:693-701, 2001
221. Russo LM, Sandoval RM, McKee M, Osicka TM, Collins AB, Brown D, Molitoris BA, Comper WD: The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: Retrieval is disrupted in nephrotic states. Kidney Int 71:504-513, 2007
222. Russo LM, Bakris GL, Comper WD: Renal handling of albumin: a critical review of basic concepts and perspective. Am J Kidney Dis 39:899-919, 2002
223. Gekle M: Renal albumin handling: A look at the dark side of the filter. Kidney Int 71:479-481, 2007
224. Comper WD, Haraldsson B, Ohlson M, Sorensson J: Charge Selectivity is a Concept That Has Yet to be Demonstrated. Am J Physiol Renal Physiol 281:F992-F993, 2001
225. Haraldsson B, Nystrom J, Deen WM: Properties of the Glomerular Barrier and Mechanisms of Proteinuria. Physiol Rev 88:451-487, 2008
226. Kriz W, Bankir L: A standard nomenclature for structure of the kidney. The Renal Commission of the International Union of Physiological Sciences(IUPS). Anat Embryol (Berl) 178:N1-N8, 1988
227. Birn H, Christensen EI: Renal albumin absorption in physiology and pathology. Kidney Int 69:440-449, 2006
228. Gekle M: Renal tubule albumin transport. Annual Review of Physiology 67:573-594, 2005
229. Woltman AM, De Fijter JW, Zuidwijk K, Vlug AG, Bajema IM, van der Kooij SW, van Ham V, van Kooten C: Quantification of dendritic cell subsets in human renal tissue under normal and pathological conditions. Kidney Int 2007
230. Soos TJ, Sims TN, Barisoni L, Lin K, Littman DR, Dustin ML, Nelson PJ: CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. Kidney Int 70:591-596, 2006
231. Iseki K, Ikemiya Y, Iseki C, Takishita S: Proteinuria and the risk of developing end-stage renal disease. Kidney Int 63:1468-1474, 2003
232. Hillege HL, Janssen WMT, Bak AAA, Diercks GFH, Grobbee DE, Crijns HJGM, Van Gilst WH, de Zeeuw D, de Jong PE: Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. Journal of Internal Medicine 249:519-526, 2001
233. Halbesma N, Kuiken DS, Brantsma AH, Bakker SJL, Wetzels JFM, De Zeeuw D, De Jong PE, Gansevoort RT, for the PREVEND Study Group: Macroalbuminuria Is a Better Risk Marker than Low Estimated GFR to Identify Individuals at Risk for Accelerated GFR Loss in Population Screening. J Am Soc Nephrol 17:2582-2590, 2006
234. Remuzzi G, Benigni A, Remuzzi A: Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest 116:288-296, 2006
235. Ruggenenti P, Schieppati A, Remuzzi G: Progression, remission, regression of chronic renal diseases. Lancet 357:1601-1608, 2001
236. De Zeeuw D: Albuminuria, not only a cardiovascular/renal risk marker, but also a target for treatment? Kidney Int 66:S2-S6, 2004
237. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA: Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 350:672-683, 2004
238. 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
239. Jeansson M, Haraldsson B: Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. Am J Physiol Renal Physiol 290:F111-F116, 2006



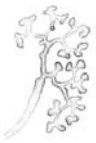
240. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R: Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. Proc Natl Acad Sci U S A 2006
241. Morello R, Zhou G, Dreyer SD, Harvey SJ, Ninomiya Y, Thorner PS, Miner JH, Cole W, Winterpacht A, Zabel B, Oberg KC, Lee B: Regulation of glomerular basement membrane collagen expression by LMX1B contributes to renal disease in nail patella syndrome. Nat Genet 27:205-208, 2001
242. Noakes PG, Miner JH, Gautam M, Cunningham JM, Sanes JR, Merlie JP: The renal glomerulus of mice lacking s-laminin/laminin [beta]2: nephrosis despite molecular compensation by laminin [beta]1. Nat Genet 10:400-406, 1995
243. Pierson M, Cordier J, Hervouuet F, Rauber G: An unusual congenital and familial congenital malformative combination involving the eye and the kidney. J Genet Hum 12:184-213, 1963
244. Zenker M, Pierson M, Jonveaux P, Reis A: Demonstration of two novel LAMB2 mutations in the original Pierson syndrome family reported 42 years ago. Am J Med Genet A 138:73-74, 2005
245. Zenker M, Aigner T, Wendler O, Tralau T, Muntefering H, Fenski R, Pitz S, Schumacher V, Royer-Pokora B, Wuhl E, Cochat P, Bouvier R, Kraus C, Mark K, Madlon H, Dotsch J, Rascher W, Maruniak-Chudek I, Lennert T, Neumann LM, Reis A: Human laminin {beta}2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. Hum Mol Genet 13:2625-2632, 2004
246. Morita H, Yoshimura A, Inui K, Ideura T, Watanabe H, Wang L, Soininen R, Tryggvason K: Heparan Sulfate of Perlecan Is Involved in Glomerular Filtration. J Am Soc Nephrol 16:1703-1710, 2005
247. van den Berg JG, Weening JJ: Role of the immune system in the pathogenesis of idiopathic nephrotic syndrome. Clin Sci (Lond) 107:125-136, 2004
248. Shalhoub RJ: Pathogenesis of lipoid nephrosis: a disorder of T-cell function. Lancet 2:556-560, 1974
249. Van Den Berg JG, Aten Jan, Chand MA, Claessen Nike, Dijkink Lise, Wijdenes John, Lakkis FG, Weening JJ: Interleukin-4 and Interleukin- 13 Act on Glomerular Visceral Epithelial Cells. J Am Soc Nephrol 11:413-422, 2000
250. Van Den Berg JG, Aten J, Annink C, Ravesloot JH, Weber E, Weening JJ: Interleukin-4 and -13 promote basolateral secretion of H+ and cathepsin L by glomerular epithelial cells. Am J Physiol Renal Physiol 282:F26-F33, 2002
251. Groffen AJ, Veerkamp JH, Monnens LA, van den Heuvel LP: Recent insights into the structure and functions of heparan sulfate proteoglycans in the human glomerular basement membrane. Nephrol Dial Transplant 14:2119-2129, 1999
252. Cheung PK, Klok PA, Baller JFW, Bakker WW: Induction of experimental proteinuria in vivo following infusion of human plasma hemopexin. Kidney Int 57:1512-1520, 2000
253. Patrakka J, Lahdenkari AT, Koskimies O, Holmberg C, Wartiovaara J, Jalanko H: The number of podocyte slit diaphragms is decreased in minimal change nephrotic syndrome. Pediatr Res 52:349-355, 2002
254. Doublier S, Ruotsalainen V, Salvidio G, Lupia E, Biancone L, Conaldi PG, Reponen P, Tryggvason K, Camussi G: Nephrin redistribution on podocytes is a potential mechanism for proteinuria in patients with primary acquired nephrotic syndrome. Am J Pathol 158:1723-1731, 2001
255. Furness PN, Hall LL, Shaw JA, Pringle JH: Glomerular expression of nephrin is decreased in acquired human nephrotic syndrome. Nephrol Dial Transplant 14:1234-1237, 1999
256. Patrakka J, Ruotsalainen V, Ketola I, Holmberg C, Heikinheimo M, Tryggvason K, Jalanko H: Expression of nephrin in pediatric kidney diseases. J Am Soc Nephrol 12:289-296, 2001
257. Srivastava T, Garola RE, Whiting JM, Alon US: Synaptopodin expression in idiopathic nephrotic syndrome of childhood. Kidney Int 59:118-125, 2001
258. Huh W, Kim DJ, Kim MK, Kim YG, Oh HY, Ruotsalainen V, Tryggvason K: Expression of nephrin in acquired human glomerular disease. Nephrol Dial Transplant 17:478-484, 2002
259. Koop K, Eikmans M, Baelde HJ, Kawachi H, De Heer E, Paul LC, Bruijn JA: Expression of podocyte-associated molecules in acquired human kidney diseases. J Am Soc Nephrol 14:2063-2071, 2003
260. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC: Pathologic classification of focal segmental glomerulosclerosis: a working proposal. Am J Kidney Dis 43:368-382, 2004
261. Thomas DB, Franceschini N, Hogan SL, ten Holder S, Jennette CE, Falk RJ, Jennette JC: Clinical and pathologic characteristics of focal segmental glomerulosclerosis pathologic variants. Kidney Int 69:920-926, 2006
262. Meyrier A: Mechanisms of disease: focal segmental glomerulosclerosis. Nat Clin Pract Nephrol 1:44-54, 2005
263. Markowitz GS, Appel GB, Fine PL, Fenves AZ, Loon NR, Jagannath S, Kuhn JA, Dratch AD, D'Agati VD: Collapsing focal segmental glomerulosclerosis following treatment with high-dose pamidronate. J Am Soc Nephrol 12:1164-1172, 2001
264. Winston JA, Bruggeman LA, Ross MD, Jacobson J, Ross L, D'Agati VD, Klotman PE, Klotman ME: Nephropathy and establishment of a renal reservoir of HIV type 1 during primary infection. N Engl J Med 344:1979-1984, 2001
265. Bruggeman LA, Ross MD, Tanji N, Cara A, Dikman S, Gordon RE, Burns GC, D'Agati VD, Winston JA, Klotman ME, Klotman PE: Renal epithelium is a previously unrecognized site of HIV-1 infection. J Am Soc Nephrol 11:2079-2087, 2000
266. Endlich N, Kress KR, Reiser J, Uttenweiler D, Kriz W, Mundel P, Endlich K: Podocytes respond to mechanical stress in vitro. J Am Soc Nephrol 12:413-422, 2001
267. Abbate M, Zoja C, Morigi M, Rottoli D, Angioletti S, Tomasoni S, Zanchi C, Longaretti L, Donadelli R, Remuzzi G: Transforming growth factor-beta1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins: a central pathway in progressive glomerulosclerosis. Am J Pathol 161:2179-2193, 2002
268. Davies DJ, Messina A, Thumwood CM, Ryan GB: Glomerular podocytic injury in protein overload proteinuria. Pathology 17:412-419, 1985
269. Kemper MJ, Wolf G, Muller-Wiefel DE: Transmission of Glomerular Permeability Factor from a Mother to Her Child. N Engl J Med 344:386-387, 2001

270. Savin VJ, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, Lovell H, Warady B, Gunwar S, Chonko AM, Artero M, Vincenti F: Circulating Factor Associated with Increased Glomerular Permeability to Albumin in Recurrent Focal Segmental Glomerulosclerosis. N Engl J Med 334:878-883, 1996
271. Coward RJ, Foster RR, Patton D, Ni L, Lennon R, Bates DO, Harper SJ, Mathieson PW, Saleem MA: Nephrotic plasma alters slit diaphragm-dependent signaling and translocates nephrin, Podocin, and CD2 associated protein in cultured human podocytes. J Am Soc Nephrol 16:629-637, 2005
272. Wei C, Moller CC, Altintas MM, Li J, Schwarz K, Zacchigna S, Xie L, Henger A, Schmid H, Rastaldi MP, Cowan P, Kretzler M, Parrilla R, Bendayan M, Gupta V, Nikolic B, Kalluri R, Carmeliet P, Mundel P, Reiser J: Modification of kidney barrier function by the urokinase receptor. Nat Med 14:55-63, 2008
273. Kriz W: The pathogenesis of 'classic' focal segmental glomerulosclerosis-lessons from rat models. Nephrol Dial Transplant 18 Suppl 6:vi39-vi44, 2003
274. Kihara I, Tsuchida S, Yaoita E, Yamamoto T, Hara M, Yanagihara T, Takada T: Podocyte detachment and epithelial cell reaction in focal segmental glomerulosclerosis with cellular variants. Kidney Int Suppl 63:S171-S176, 1997
275. Nagata M, Hattori M, Hamano Y, Ito K, Saitoh K, Watanabe T: Origin and phenotypic features of hyperplastic epithelial cells in collapsing glomerulopathy. Am J Kidney Dis 32:962-969, 1998
276. Remuzzi G, Schieppati A, Ruggenenti P: Nephropathy in Patients with Type 2 Diabetes. N Engl J Med 346:1145-1151, 2002
277. Wolf G, Chen S, Ziyadeh FN: From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. Diabetes 54:1626-1634, 2005
278. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, Meyer TW: Podocyte loss and progressive glomerular injury in type II diabetes. J Clin Invest 99:342-348, 1997
279. Iglesias-de la Cruz MC, Ziyadeh FN, Isono M, Kouahou M, Han DC, Kalluri R, Mundel P, Chen S: Effects of high glucose and TGF-beta1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. Kidney Int 62:901-913, 2002
280. Wolf G: New insights into the pathophysiology of diabetic nephropathy: from haemodynamics to molecular pathology. European Journal of Clinical Investigation 34:785-796, 2004
281. Wendt TM, Tanji N, Guo J, Kislinger TR, Qu W, Lu Y, Bucciarelli LG, Rong LL, Moser B, MARKOWITZ GS, Stein G, Bierhaus A, Liliensiek B, Arnold B, Nawroth PP, Stern DM, D'AGATI VD, Schmidt AM: RAGE Drives the Development of Glomerulosclerosis and Implicates Podocyte Activation in the Pathogenesis of Diabetic Nephropathy. Am J Pathol 162:1123-1137, 2003
282. Chen S, Lee JS, Iglesias-de la Cruz MC, Wang A, Izquierdo-Lahuerta A, Gandhi NK, Danesh FR, Wolf G, Ziyadeh FN: Angiotensin II stimulates {alpha}3(IV) collagen production in mouse podocytes via TGF-{beta} and VEGF signalling: implications for diabetic glomerulopathy. Nephrol Dial Transplant 2005
283. Benigni A, Gagliardini E, Remuzzi G: Changes in glomerular perm-selectivity induced by angiotensin II imply podocyte dysfunction and slit diaphragm protein rearrangement. Semin Nephrol 24:131-140, 2004
284. Chen HC, Chen CA, Guh JY, Chang JM, Shin SJ, Lai YH: Altering expression of [alpha]3[beta]1 integrin on podocytes of human and rats with diabetes. Life Sciences 67:2345-2353, 2000
285. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, Koide H: Urinary excretion of podocytes in patients with diabetic nephropathy. Nephrol Dial Transplant 15:1379-1383, 2000
286. Patari A, Forsblom C, Havana M, Taipale H, Groop PH, Holthofer H: Nephrinuria in diabetic nephropathy of type 1 diabetes. Diabetes 52:2969-2974, 2003
287. Ding G, Reddy K, Kapasi AA, Franki N, Gibbons N, Kasinath BS, Singhal PC: Angiotensin II induces apoptosis in rat glomerular epithelial cells. Am J Physiol Renal Physiol 283:F173-F180, 2002
288. Langham RG, Kelly DJ, Cox AJ, Thomson NM, Holthofer H, Zaoui P, Pinel N, Cordonnier DJ, Gilbert RE: Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy: effects of angiotensin converting enzyme inhibition. Diabetologia 45:1572-1576, 2002
289. Xu ZG, Ryu DR, Yoo TH, Jung DS, Kim JJ, Kim HJ, Choi HY, Kim JS, Adler SG, Natarajan R, Han DS, Kang SW: P-Cadherin is decreased in diabetic glomeruli and in glucose-stimulated podocytes in vivo and in vitro studies. Nephrol Dial Transplant 20:524-531, 2005
290. Couser WG: Membranous Nephropathy: A Long Road but Well Traveled. J Am Soc Nephrol 16:1184-1187, 2005
291. Kerjaschki D, Neale TJ: Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis) [editorial]. J Am Soc Nephrol 7:2518-2526, 1996
292. Pippin JW, Durvasula R, Petermann A, Hiromura K, Couser WG, Shankland SJ: DNA damage is a novel response to sublytic complement C5b-9-induced injury in podocytes. J Clin Invest 111:877-885, 2003
293. Nangaku M, Shankland SJ, Couser WG: Cellular response to injury in membranous nephropathy. J Am Soc Nephrol 16:1195-1204, 2005
294. Nakatsue T, Koike H, Han GD, Suzuki K, Miyauchi N, Yuan H, Salant DJ, Gejyo F, Shimizu F, Kawachi H: Nephrin and podocin dissociate at the onset of proteinuria in experimental membranous nephropathy. Kidney Int 67:2239-2253, 2005
295. Yuan H, Takeuchi E, Taylor GA, McLaughlin M, Brown D, Salant DJ: Nephrin dissociates from actin, and its expression is reduced in early experimental membranous nephropathy. J Am Soc Nephrol 13:946-956, 2002
296. Fischer E, Mougenot B, Callard P, Ronco P, Rossert J: Abnormal expression of glomerular basement membrane laminins in membranous glomerulonephritis. Nephrol Dial Transplant 15:1956-1964, 2000



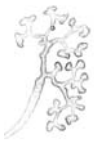
297. Haas M, Mayer G, Wirnsberger G, Holzer H, Ratschek M, Neyer U, Neuweiler J, Kramar R, Schneider B, Breiteneder-Geleff S, Regele HM, Horl WH, Kerjaschki D: Antioxidant treatment of therapy-resistant idiopathic membranous nephropathy with probucol: a pilot study. Wien Klin Wochenschr 114:143-147, 2002
298. Shirato I: Podocyte process effacement in vivo. Microsc Res Tech 57:241-246, 2002
299. 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
300. Ichikawa I, Ma J, Motojima M, Matsusaka T: Podocyte damage damages podocytes: autonomous vicious cycle that drives local spread of glomerular sclerosis. Curr Opin Nephrol Hypertens 14:205-210, 2005
301. Zandi-Nejad K, Eddy AA, Glassock RJ, Brenner BM: Why is proteinuria an ominous biomarker of progressive kidney disease? Kidney Int SupplS76-S89, 2004
302. Gross ML, Hanke W, Koch A, Ziebart H, Amann K, Ritz E: Intraperitoneal protein injection in the axolotl: The amphibian kidney as a novel model to study tubulointerstitial activation. Kidney Int 62:51-59, 2002
303. Kriz W, LeHir M: Pathways to nephron loss starting from glomerular diseases-insights from animal models. Kidney Int 67:404-419, 2005
304. Brunskill NJ: Albumin Signals the Coming of Age of Proteinuric Nephropathy. J Am Soc Nephrol 15:504-505, 2004
305. Reich H, Tritchler D, Herzenberg AM, Kassiri Z, Zhou X, Gao W, Scholey JW: Albumin Activates ERK Via EGF Receptor in Human Renal Epithelial Cells. J Am Soc Nephrol 16:1266-1278, 2005
306. Wang Y, Rangan GK, Tay YC, Wang Y, Harris DC: Induction of monocyte chemoattractant protein-1 by albumin is mediated by nuclear factor kappaB in proximal tubule cells. J Am Soc Nephrol 10:1204-1213, 1999
307. Tang S, Leung JCK, Abe K, Chan KW, Chan LYY, Chan TM, Lai KN: Albumin stimulates interleukin-8 expression in proximal tubular epithelial cells in vitro and in vivo. J Clin Invest 111:515-527, 2003
308. Nakajima H, Takenaka M, Kaimori JY, Hamano T, Iwatani H, Sugaya T, Ito T, Hori M, Imai E: Activation of the Signal Transducer and Activator of Transcription Signaling Pathway in Renal Proximal Tubular Cells by Albumin. J Am Soc Nephrol 15:276-285, 2004
309. Zoja C, Donadelli R, Colleoni S, Figliuzzi M, Bonazzola S, Morigi M, Remuzzi G: Protein overload stimulates RANTES production by proximal tubular cells depending on NF-kappa B activation. Kidney Int 53:1608-1615, 1998
310. Zoja C, Morigi M, Figliuzzi M, Bruzzi I, Oldroyd S, Benigni A, Ronco P, Remuzzi G: Proximal tubular cell synthesis and secretion of endothelin-1 on challenge with albumin and other proteins. Am J Kidney Dis 26:934-941, 1995
311. Wohlfarth V, Drumm K, Mildenberger S, Freudinger R, Gekle M: Protein uptake disturbs collagen homeostasis in proximal tubule-derived cells. Kidney Int SupplS103-S109, 2003
312. Yard BA, Chorianopoulos E, Herr D, van der Woude FJ: Regulation of endothelin-1 and transforming growth factor-{beta}1 production in cultured proximal tubular cells by albumin and heparan sulphate glycosaminoglycans. Nephrol Dial Transplant 16:1769-1775, 2001
313. Stephan JP, Mao W, Filvaroff E, Cai L, Rabkin R, Pan G: Albumin stimulates the accumulation of extracellular matrix in renal tubular epithelial cells. Am J Nephrol 24:14-19, 2004
314. Largo R, Gomez-Garre D, Soto K, Marron B, Blanco J, Gazapo RM, Plaza JJ, Egido J: Angiotensin-Converting Enzyme Is Upregulated in the Proximal Tubules of Rats With Intense Proteinuria. Hypertension 33:732-739, 1999
315. Thomas ME, Brunskill NJ, Harris KPG, Bailey E, Pringle JH, Furness PN, Walls J: Proteinuria induces tubular cell turnover: A potential mechanism for tubular atrophy. Kidney Int 55:890-898, 1999
316. Hirschberg R, Wang S: Proteinuria and growth factors in the development of tubulointerstitial injury and scarring in kidney disease. Curr Opin Nephrol Hypertens 14:43-52, 2005
317. Burton CJ, Combe C, Walls J, Harris KPG: Secretion of chemokines and cytokines by human tubular epithelial cells in response to proteins. Nephrol Dial Transplant 14:2628-2633, 1999
318. Burton CJ, Combe C, Walls J, Harris KP: Fibronectin production by human tubular cells: the effect of apical protein. Kidney Int 50:760-767, 1996
319. Arici M, Brown J, Williams M, Harris KPG, Walls J, Brunskill NJ: Fatty acids carried on albumin modulate proximal tubular cell fibronectin production: a role for protein kinase C. Nephrol Dial Transplant 17:1751-1757, 2002
320. Arici M, Chana R, Lewington A, Brown J, Brunskill NJ: Stimulation of Proximal Tubular Cell Apoptosis by Albumin-Bound Fatty Acids Mediated by Peroxisome Proliferator Activated Receptor-{gamma}. J Am Soc Nephrol 14:17-27, 2003
321. Joles JA, Kunter U, Janssen U, Kriz W, Rabelink TJ, Koomans HA, Floege J: Early mechanisms of renal injury in hypercholesterolemic or hypertriglyceridemic rats. J Am Soc Nephrol 11:669-683, 2000
322. Abbate M, Zoja C, Remuzzi G: How does proteinuria cause progressive renal damage? J Am Soc Nephrol 17:2974-2984, 2006
323. Perico N, Codreanu I, Schieppati A, Remuzzi G: Pathophysiology of disease progression in proteinuric nephropathies. Kidney Int 67:S79-S82, 2005
324. Stehouwer CDA, Smulders YM: Microalbuminuria and Risk for Cardiovascular Disease: Analysis of Potential Mechanisms. J Am Soc Nephrol 17:2106-2111, 2006
325. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A: Albuminuria reflects widespread vascular damage. Diabetologia 32:219-226, 1989
326. Frishberg Y, Feinstein S, Rinat C, Becker-Cohen R, Lerer I, Raas-Rothschild A, Ferber B, Nir A: The Heart of Children with Steroid-Resistant Nephrotic Syndrome: Is It All Podocin? J Am Soc Nephrol 17:227-231, 2006
327. Moreira-Rodrigues M, Roncon-Albuquerque R, Jr., Henriques-Coelho T, Lourenco AP, Sampaio-Maia B, Santos J, Pestana M, Leite-Moreira AF: Cardiac remodeling and dysfunction in nephrotic syndrome. Kidney Int 2007

328. Halimi JM, Laouad I, Buchler M, Al Najjar A, Chatelet V, Houssaini TS, Nivet H, Lebranchu Y: Early low-grade proteinuria: causes, short-term evolution and long-term consequences in renal transplantation. Am J Transplant 5:2281-2288, 2005
329. Hohage H, Kleyer U, Bruckner D, August C, Zidek W, Spieker C: Influence of proteinuria on long-term transplant survival in kidney transplant recipients. Nephron 75:160-165, 1997
330. Reichel H, Zeier M, Ritz E: Proteinuria after renal transplantation: pathogenesis and management. Nephrol Dial Transplant 19:301-305, 2004
331. Zayas CF, Guasch A: Early glomerular dysfunction in human renal allografts. Kidney Int 60:1938-1947, 2001
332. Taal MW, Brenner BM: Predicting initiation and progression of chronic kidney disease: Developing renal risk scores. Kidney Int 70:1694-1705, 2006
333. Haroun MK, Jaar BG, Hoffman SC, Comstock GW, Klag MJ, Coresh J: Risk Factors for Chronic Kidney Disease: A Prospective Study of 23,534 Men and Women in Washington County, Maryland. J Am Soc Nephrol 14:2934-2941, 2003
334. Opelz G, Dohler B: Improved long-term outcomes after renal transplantation associated with blood pressure control. Am J Transplant 5:2725-2731, 2005
335. Risdon RA, Sloper JC, De Wardener HE: Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. The Lancet 292:363-366, 1968
336. D'Amico G: Tubulo-interstitial damage in glomerular diseases: its role in the progression of the renal damage. Nephrol Dial Transplant 13:80-85, 1998
337. Harris RC, Neilson EG: Toward a unified theory of renal progression. Annual Review of Medicine 57:365-380, 2006
338. Kriz W, Hosser H, Hahnel B, Gretz N, Provoost A: From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies. Nephrol Dial Transplant 13:2781-2798, 1998
339. Kriz W, Elger M, Hosser H, Hahnel B, Provoost A, Kranzlin B, Gretz N: How does podocyte damage result in tubular damage? Kidney Blood Press Res 22:26-36, 1999
340. Kriz W, Hartmann I, Hosser H, Hahnel B, Kranzlin B, Provoost A, Gretz N: Tracer studies in the rat demonstrate misdirected filtration and peritubular filtrate spreading in nephrons with segmental glomerulosclerosis. J Am Soc Nephrol 12:496-506, 2001
341. Pichler R, Giachelli C, Young B, Alpers CE, Couser WG, Johnson RJ: The pathogenesis of tubulointerstitial disease associated with glomerulonephritis: the glomerular cytokine theory. Miner Electrolyte Metab 21:317-327, 1995
342. Kang DH, Kanellis J, Hugo C, Truong L, Anderson S, Kerjaschki D, Schreiner GF, Johnson RJ: Role of the Microvascular Endothelium in Progressive Renal Disease. J Am Soc Nephrol 13:806-816, 2002
343. Lindenmeyer MT, Kretzler M, Boucherot A, Berra S, Yasuda Y, Henger A, Eichinger F, Gaiser S, Schmid H, Rastaldi MP, Schrier RW, Schlondorff D, Cohen CD: Interstitial Vascular Rarefaction and Reduced VEGF-A Expression in Human Diabetic Nephropathy. J Am Soc NephrolASN, 2007
344. Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ: Impaired Angiogenesis in the Remnant Kidney Model: II. Vascular Endothelial Growth Factor Administration Reduces Renal Fibrosis and Stabilizes Renal Function. J Am Soc Nephrol 12:1448-1457, 2001
345. Nangaku M: Chronic Hypoxia and Tubulointerstitial Injury: A Final Common Pathway to End-Stage Renal Failure. J Am Soc Nephrol 17:17-25, 2006
346. Healy E, Brady HR: Role of tubule epithelial cells in the pathogenesis of tubulointerstitial fibrosis induced by glomerular disease. Curr Opin Nephrol Hypertens 7:525-530, 1998
347. Nath KA, Hostetter MK, Hostetter TH: Pathophysiology of chronic tubulo-interstitial disease in rats. Interactions of dietary acid load, ammonia, and complement component C3. J Clin Invest 76:667-675, 1985
348. Anders HJ, Vielhauer V, Schlondorff D: Chemokines and chemokine receptors are involved in the resolution or progression of renal disease. Kidney Int 63:401-415, 2003
349. van Kooten C, Daha MR: Cytokine cross-talk between tubular epithelial cells and interstitial immunocompetent cells. Curr Opin Nephrol Hypertens 10:55-59, 2001
350. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG: Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest 110:341-350, 2002
351. Liu Y: Epithelial to Mesenchymal Transition in Renal Fibrogenesis: Pathologic Significance, Molecular Mechanism, and Therapeutic Intervention. J Am Soc Nephrol 15:1-12, 2004
352. Schnaper HW, Kopp JB: Why kidneys fail: report from an American Society of Nephrology Advances in Research Conference. J Am Soc Nephrol 17:1777-1781, 2006
353. Iwano M, Neilson EG: Mechanisms of tubulointerstitial fibrosis. Curr Opin Nephrol Hypertens 13:279-284, 2004
354. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 307:652-659, 1982
355. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM: Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am J Physiol Renal Physiol 241:F85-F93, 1981
356. Toledo-Pereyra LH, Palma-Vargas JM: Searching for history in transplantation: early modern attempts at surgical kidney grafting. Transplant Proc 31:2945-2948, 1999
357. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Pentlow BD, Rolles K: Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet 2:1323-1327, 1978
358. Gjertson DW: Survival trends in long-term first cadaver-donor kidney transplants. Clin Transpl225-235, 1991



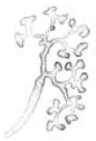
359. Meier-Kriesche HU, Schold JD, Kaplan B: Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? Am J Transplant 4:1289-1295, 2004
360. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B: Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant 4:378-383, 2004
361. Chapman JR, O'Connell PJ, Nankivell BJ: Chronic renal allograft dysfunction. J Am Soc Nephrol 16:3015-3026, 2005
362. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D: Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 342:605-612, 2000
363. Sijpkens YWJ, Joosten SA, Paul LC: Chronic rejection in renal transplantation. Transplantation Reviews 17:117-130, 2003
364. Colvin RB: Chronic allograft nephropathy. N Engl J Med 349:2288-2290, 2003
365. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nickeleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madriga L, Salomon DR, Seron D, Sheaff M, Weening JJ: Banff '05 Meeting Report: Differential Diagnosis of Chronic Allograft Injury and Elimination of Chronic Allograft Nephropathy (CAN). American Journal of Transplantation 7:518-526, 2007
366. Paul LC: Chronic allograft nephropathy: An update. Kidney Int 56:783-793, 1999
367. Joosten SA, Sijpkens YW, van Kooten C, Paul LC: Chronic renal allograft rejection: pathophysiologic considerations. Kidney Int 68:1-13, 2005
368. Gourishankar S, Melk A, Halloran P: Nonimmune Mechanisms of Injury in Renal Transplantation. Transplantation Reviews 16:73-86, 2002
369. Halloran PF, Melk A, Barth C: Rethinking chronic allograft nephropathy: the concept of accelerated senescence. J Am Soc Nephrol 10:167-181, 1999
370. Jindal RM, Hariharan S: Chronic rejection in kidney transplants. An in-depth review. Nephron 83:13-24, 1999
371. Paul LC: Current knowledge of the pathogenesis of chronic allograft dysfunction. Transplant Proc 31:1793-1795, 1999
372. van der Woude FJ: Graft immunogenicity revisited: relevance of tissue-specific immunity, brain death and donor pretreatment. Nephron 91:181-187, 2002
373. Takemoto SK, Terasaki PI, Gjertson DW, Cecka JM: Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. N Engl J Med 343:1078-1084, 2000
374. Smits JM, van Houwelingen HC, De Meester J, le Cessie S, Persijn GG, Claas FH, Frei U: Permanent detrimental effect of nonimmunological factors on long-term renal graft survival: a parsimonious model of time-dependency. Transplantation 70:317-323, 2000
375. Morris PJ, Johnson RJ, Fuggle SV, Belger MA, Briggs JD: Analysis of factors that affect outcome of primary cadaveric renal transplantation in the UK. HLA Task Force of the Kidney Advisory Group of the United Kingdom Transplant Support Service Authority (UKTSSA). Lancet 354:1147-1152, 1999
376. Opelz G, Wujciak T, Dohler B, Scherer S, Mytilineos J: HLA compatibility and organ transplant survival. Collaborative Transplant Study. Rev Immunogenet 1:334-342, 1999
377. Su X, Zenios SA, Chakkera H, Milford EL, Chertow GM: Diminishing significance of HLA matching in kidney transplantation. Am J Transplant 4:1501-1508, 2004
378. Meier-Kriesche HU, Ojo AO, Leichtman AB, Magee JC, Rudich SM, Hanson JA, Cibrik DM, Kaplan B: Interaction of mycophenolate mofetil and HLA matching on renal allograft survival. Transplantation 71:398-401, 2001
379. McKenna RM, Takemoto SK, Terasaki PI: Anti-HLA antibodies after solid organ transplantation. Transplantation 69:319-326, 2000
380. Piazza A, Poggi E, Borrelli L, Servetti S, Monaco PI, Buonomo O, Valeri M, Torlone N, Adorno D, Casciani CU: Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation: posttransplant analysis using flow cytometric techniques. Transplantation 71:1106-1112, 2001
381. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, Cecka MJ, Cosyns JP, Demetris AJ, Fishbein MC, Fogo A, Furness P, Gibson IW, Glotz D, Hayry P, Hunsickern L, Kashgarian M, Kerman R, Magil AJ, Montgomery R, Morozumi K, Nickeleit V, Randhawa P, Regele H, Seron D, Seshan S, Sund S, Trpkov K: Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection. Am J Transplant 3:708-714, 2003
382. Butler JA, Roderick P, Mullee M, Mason JC, Peveler RC: Frequency and impact of nonadherence to immunosuppressants after renal transplantation: a systematic review. Transplantation 77:769-776, 2004
383. Sijpkens YW, Doxiadis II, De Fijter JW, Mallat MJ, van Es LA, De Lange P, Zwinderman AH, Westendorp RG, van Kemenade FJ, Bruijn JA, Claas FH, Paul LC: Sharing cross-reactive groups of MHC class I improves long-term graft survival. Kidney Int 56:1920-1927, 1999
384. Leggat JE, Jr., Ojo AO, Leichtman AB, Port FK, Wolfe RA, Turenne MN, Held PJ: Long-term renal allograft survival: prognostic implication of the timing of acute rejection episodes. Transplantation 63:1268-1272, 1997
385. Meier-Kriesche HU, Ojo AO, Hanson JA, Cibrik DM, Punch JD, Leichtman AB, Kaplan B: Increased impact of acute rejection on chronic allograft failure in recent era. Transplantation 70:1098-1100, 2000
386. Massy ZA, Guijarro C, Wiederkehr MR, Ma JZ, Kasiske BL: Chronic renal allograft rejection: immunologic and nonimmunologic risk factors. Kidney Int 49:518-524, 1996
387. Shishido S, Asanuma H, Nakai H, Mori Y, Satoh H, Kamimaki I, Hataya H, Ikeda M, Honda M, Hasegawa A: The impact of repeated subclinical acute rejection on the progression of chronic allograft nephropathy. J Am Soc Nephrol 14:1046-1052, 2003

388. Joseph JT, Kingsmore DB, Junor BJ, Briggs JD, Mun WY, Jaques BC, Hamilton DN, Jardine AG, Jindal RM: The impact of late acute rejection after cadaveric kidney transplantation. Clin Transplant 15:221-227, 2001
389. Sijpkens YW, Doxiadis II, Mallat MJ, De Fijter JW, Bruijn JA, Claas FH, Paul LC: Early versus late acute rejection episodes in renal transplantation. Transplantation 75:204-208, 2003
390. van Saase JL, van der Woude FJ, Thorogood J, Hollander AA, van Es LA, Weening JJ, van Bockel JH, Bruijn JA: The relation between acute vascular and interstitial renal allograft rejection and subsequent chronic rejection. Transplantation 59:1280-1285, 1995
391. Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB: Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. Nat Med 5:1298-1302, 1999
392. Gauthier P, Helderman JH: Cyclosporine avoidance. J Am Soc Nephrol 11:1933-1936, 2000
393. Gourishankar S, Jhangri GS, Cockfield SM, Halloran PF: Donor tissue characteristics influence cadaver kidney transplant function and graft survival but not rejection. J Am Soc Nephrol 14:493-499, 2003
394. Terasaki PI, Gjertson DW, Cecka JM, Takemoto S, Cho YW: Significance of the donor age effect on kidney transplants. Clin Transplant 11:366-372, 1997
395. Smits JM, De Meester J, Persijn GG, Claas FH, Vanrenterghem Y: Long-term results of solid organ transplantation. Report from the Eurotransplant International Foundation. Clin Transpl109-127, 1996
396. Prommool S, Jhangri GS, Cockfield SM, Halloran PF: Time dependency of factors affecting renal allograft survival. J Am Soc Nephrol 11:565-573, 2000
397. Gjertson DW: Explainable variation in renal transplant outcomes: a comparison of standard and expanded criteria donors. Clin Transpl303-314, 2004
398. De Fijter JW, Mallat MJ, Doxiadis II, Ringers J, Rosendaal FR, Claas FH, Paul LC: Increased immunogenicity and cause of graft loss of old donor kidneys. J Am Soc Nephrol 12:1538-1546, 2001
399. Kim MS, Kim DK, Myoung SM, Kim SI, Oh CK, Kim YS, Lee JH, Kang SW, Park K: Chronologically different impacts of immunologic and non-immunologic risk factors on renal allograft function. Clin Transplant 19:742-750, 2005
400. Pape L, Hoppe J, Becker T, Ehrich JH, Neipp M, Ahlenstiel T, Offner G: Superior long-term graft function and better growth of grafts in children receiving kidneys from paediatric compared with adult donors. Nephrol Dial Transplant 21:2596-2600, 2006
401. Meier-Kriesche HU, Ojo AO, Leavey SF, Hanson JA, Leichtman AB, Magee JC, Cibrik DM, Kaplan B: Gender differences in the risk for chronic renal allograft failure. Transplantation 71:429-432, 2001
402. Meier-Kriesche HU, Cibrik DM, Ojo AO, Hanson JA, Magee JC, Rudich SM, Leichtman AB, Kaplan B: Interaction between donor and recipient age in determining the risk of chronic renal allograft failure. J Am Geriatr Soc 50:14-17, 2002
403. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S: High Survival Rates of Kidney Transplants from Spousal and Living Unrelated Donors. N Engl J Med 333:333-336, 1995
404. Koo DD, Welsh KI, McLaren AJ, Roake JA, Morris PJ, Fuggle SV: Cadaver versus living donor kidneys: impact of donor factors on antigen induction before transplantation. Kidney Int 56:1551-1559, 1999
405. Ojo AO, Wolfe RA, Held PJ, Port FK, Schmouder RL: Delayed graft function: risk factors and implications for renal allograft survival. Transplantation 63:968-974, 1997
406. Perico N, Cattaneo D, Sayegh MH, Remuzzi G: Delayed graft function in kidney transplantation. Lancet 364:1814-1827, 2004
407. Giral-Classe M, Hourmant M, Cantarovich D, Dantal J, Blancho G, Daguin P, Ancelet D, Soulillou JP: Delayed graft function of more than six days strongly decreases long-term survival of transplanted kidneys. Kidney Int 54:972-978, 1998
408. Cosio FG, Pelletier RP, Falkenhain ME, Henry ML, Elkhammas EA, Davies EA, Bumgardner GL, Ferguson RM: Impact of acute rejection and early allograft function on renal allograft survival. Transplantation 63:1611-1615, 1997
409. Lopez-Hoyos M, Fernandez-Fresnedo G, Rodrigo E, Ruiz JC, Arias M: Effect of Delayed Graft Function in Hypersensitized Kidney Transplant Recipients. Human Immunology 66:371-377, 2005
410. Seeman T, imkova E, Kreisinger J, Vondrak K, ek J, ak P, Janda J: Proteinuria in Children After Renal Transplantation. Transplantation Proceedings 37:4282-4283, 2005
411. Roodnat JI, Mulder PG, Rischen-Vos J, van Riemsdijk IC, van Gelder T, Zietse R, IJzermans JN, Weimar W: Proteinuria after renal transplantation affects not only graft survival but also patient survival. Transplantation 72:438-444, 2001
412. Arias M, Fernandez-Fresnedo G, Rodrigo E, Ruiz JC, Gonzalez-Cotorruelo J, Gomez-Alamillo C: Non-immunologic intervention in chronic allograft nephropathy. Kidney Int SupplS118-S123, 2005
413. Fernandez-Fresnedo G, Plaza JJ, Sanchez-Plumed J, Sanz-Guajardo A, Palomar-Fontanet R, Arias M: Proteinuria: a new marker of long-term graft and patient survival in kidney transplantation. Nephrol Dial Transplant 19 Suppl 3:iii47-iii51, 2004
414. Paul LC, Sijpkens YWJ, de Fijter JW: Calcineurin inhibitors and chronic renal allograft dysfunction: Not enough or too much? Transplantation Reviews 15:82-92, 2001
415. Peschke B, Scheuermann EH, Geiger H, Bolscher S, Kachel HG, Lenz T: Hypertension is associated with hyperlipidemia, coronary heart disease and chronic graft failure in kidney transplant recipients. Clin Nephrol 51:290-295, 1999
416. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB: Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol 12:574-582, 2001
417. Racusen LC, Solez K, Colvin R: Fibrosis and atrophy in the renal allograft: interim report and new directions. Am J Transplant 2:203-206, 2002



418. Rowshani AT, Scholten EM, Bemelman F, Eikmans M, Idu M, Roos-van Groningen MC, Surachno JS, Mallat MJ, Paul LC, De Fijter JW, Bajema IM, ten B, I, Florquin S: No difference in degree of interstitial Sirius red-stained area in serial biopsies from area under concentration-over-time curves-guided cyclosporine versus tacrolimus-treated renal transplant recipients at one year. J Am Soc Nephrol 17:305-312, 2006
419. Baboolal K, Jones GA, Janezic A, Griffiths DR, Jurewicz WA: Molecular and structural consequences of early renal allograft injury. Kidney Int 61:686-696, 2002
420. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR: The natural history of chronic allograft nephropathy. N Engl J Med 349:2326-2333, 2003
421. Scholten EM, Rowshani AT, Cremers S, Bemelman FJ, Eikmans M, van Kan E, Mallat MJ, Florquin S, Surachno J, ten Berge IJ, Bajema IM, de Fijter JW: Untreated Rejection in 6-Month Protocol Biopsies Is Not Associated with Fibrosis in Serial Biopsies or with Loss of Graft Function. J Am Soc Nephrol 17:2622-2632, 2006
422. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, Drachenberg CB, Fogo AB, Furness P, Gaber LW, Gibson IW, Glotz D, Goldberg JC, Grande J, Halloran PF, Hansen HE, Hartley B, Hayry PJ, Hill CM, Hoffman EO, Hunsicker LG, Lindblad AS, Yamaguchi Y, .: The Banff 97 working classification of renal allograft pathology. Kidney Int 55:713-723, 1999
423. Ivanyi B: Transplant capillaropathy and transplant glomerulopathy: ultrastructural markers of chronic renal allograft rejection. Nephrol Dial Transplant 18:655-660, 2003
424. Colvin RB, Smith RN: Antibody-mediated organ-allograft rejection. Nat Rev Immunol 5:807-817, 2005
425. Racusen LC, Haas M: Antibody-Mediated Rejection in Renal Allografts: Lessons from Pathology. Clin J Am Soc Nephrol 1:415-420, 2006
426. Solez K, Vincenti F, Filo RS: Histopathologic findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tarolimus versus cyclosporine: a report of the FK506 Kidney Transplant Study Group. Transplantation 66:1736-1740, 1998
427. Mihatsch MJ, Thiel G, Ryffel B: Histopathology of cyclosporine nephrotoxicity. Transplant Proc 20:759-771, 1988
428. Mihatsch MJ, Antonovych T, Bohman SO, Habib R, Helmchen U, Noel LH, Olsen S, Sibley RK, Kemeny E, Feutren G: Cyclosporin A nephropathy: standardization of the evaluation of kidney biopsies. Clin Nephrol 41:23-32, 1994
429. Mihatsch MJ, Morozumi K, Strom EH, Ryffel B, Gudat F, Thiel G: Renal transplant morphology after long-term therapy with cyclosporine. Transplant Proc 27:39-42, 1995
430. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M: Cyclosporine-associated chronic nephropathy. N Engl J Med 311:699-705, 1984
431. Sijpkens YW, Mallat MJ, Siegert CE, Zwinderman AH, Westendorp RG, De Fijter JW, van Es LA, Paul LC: Risk factors of cyclosporine nephrotoxicity after conversion from Sandimmune to Neoral. Clin Nephrol 55:149-155, 2001
432. Nankivell BJ, Chapman JR: Chronic allograft nephropathy: current concepts and future directions. Transplantation 81:643-654, 2006
433. Liptak P, Ivanyi B: Primer: Histopathology of calcineurin-inhibitor toxicity in renal allografts. Nat Clin Pract Nephrol 2:398-404, 2006
434. Sis B, Dadras F, Khoshjou F, Cockfield S, Mihatsch MJ, Solez K: Reproducibility studies on arteriolar hyaline thickening scoring in calcineurin inhibitor-treated renal allograft recipients. Am J Transplant 6:1444-1450, 2006
435. Hauer HA, Bajema IM, De Heer E, Hermans J, Hagen EC, Bruijn JA: Distribution of renal lesions in idiopathic systemic vasculitis: A three-dimensional analysis of 87 glomeruli. Am J Kidney Dis 36:257-265, 2000
436. Furness PN, Taub N, Assmann KJ, Banfi G, Cosyns JP, Dorman AM, Hill CM, Kapper SK, Waldherr R, Laurinavicius A, Marcussen N, Martins AP, Nogueira M, Regele H, Seron D, Carrera M, Sund S, Taskinen EI, Paavonen T, Tihomirova T, Rosenthal R: International variation in histologic grading is large, and persistent feedback does not improve reproducibility. Am J Surg Pathol 27:805-810, 2003
437. Furness PN, Taub N: International variation in the interpretation of renal transplant biopsies: report of the CERTPAP Project. Kidney Int 60:1998-2012, 2001
438. Chapman J: Scoring arteriolar hyalinosis in renal allograft biopsies: so important and yet so tricky. Nat Clin Pract Nephrol 2:622-623, 2006
439. Mihatsch M, Ryffel B, Gudat F: The differential diagnosis between rejection and cyclosporin toxicity. Kidney Int 48:S-63-S-69, 1995
440. Kerjaschki D, Huttary N, Raab I, Regele H, Bojarski-Nagy K, Bartel G, Krober SM, Greinix H, Rosenmaier A, Karlhofer F, Wick N, Mazal PR: Lymphatic endothelial progenitor cells contribute to de novo lymphangiogenesis in human renal transplants. Nat Med 12:230-234, 2006
441. Kerjaschki D: The crucial role of macrophages in lymphangiogenesis. J Clin Invest 115:2316-2319, 2005
442. Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, Masek M, Salvatierra O, Jr.: Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. N Engl J Med 349:125-138, 2003
443. Joosten SA, Sijpkens YW, van H, V, Trouw LA, van d, V, van den HB, van Kooten C, Paul LC: Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy. Am J Transplant 5:383-393, 2005
444. Bakker RC, Scholten EM, de Fijter JW, Paul LC: Chronic cyclosporine nephrotoxicity in renal transplantation. Transplantation Reviews 18:54-64, 2004
445. Busauschina A, Schnuelle P, van der Woude FJ: Cyclosporine nephrotoxicity. Transplant Proc 36:229S-233S, 2004

446. Pankewycz OG, Miao L, Isaacs R, Guan J, Pruett T, Haussmann G, Sturgill BC: Increased renal tubular expression of transforming growth factor beta in human allografts correlates with cyclosporine toxicity. Kidney Int 50:1634-1640, 1996
447. Shin GT, Khanna A, Ding R, Sharma VK, Lagman M, Li B, Suthanthiran M: In vivo expression of transforming growth factor-beta1 in humans: stimulation by cyclosporine. Transplantation 65:313-318, 1998
448. Khanna A, Plummer M, Bromberek C, Bresnahan B, Hariharan S: Expression of TGF-beta and fibrogenic genes in transplant recipients with tacrolimus and cyclosporine nephrotoxicity. Kidney Int 62:2257-2263, 2002
449. Benigni A, Bruzzi I, Mister M, Azzollini N, Gaspari F, Perico N, Gotti E, Bertani T, Remuzzi G: Nature and mediators of renal lesions in kidney transplant patients given cyclosporine for more than one year. Kidney Int 55:674-685, 1999
450. Oleggini R, Musante L, Menoni S, Botti G, Duca MD, Prudenziati M, Carrea A, Ravazzolo R, Ghiggeri GM: Characterization of a DNA binding site that mediates the stimulatory effect of cyclosporin-A on type III collagen expression in renal cells. Nephrol Dial Transplant 15:778-785, 2000
451. Islam M, Burke JF, Jr., McGowan TA, Zhu Y, Dunn SR, McCue P, Kanalas J, Sharma K: Effect of anti-transforming growth factor-beta antibodies in cyclosporine-induced renal dysfunction. Kidney Int 59:498-506, 2001
452. McMorrow T, Gaffney MM, Slattery C, Campbell E, Ryan MP: Cyclosporine A induced epithelial-mesenchymal transition in human renal proximal tubular epithelial cells. Nephrol Dial Transplant 20:2215-2225, 2005
453. Gardner SD, Field AM, Coleman DV, Hulme B: New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1:1253-1257, 1971
454. Tilney NL: Chronic rejection and its risk factors. Transplant Proc 31:41S-44S, 1999
455. Nickeleit V, Mihatsch MJ: Polyomavirus nephropathy in native kidneys and renal allografts: an update on an escalating threat. Transplant International 19:960-973, 2006
456. Melk A, Schmidt BM, Vongwiwatana A, Rayner DC, Halloran PF: Increased expression of senescence-associated cell cycle inhibitor p16INK4a in deteriorating renal transplants and diseased native kidney. Am J Transplant 5:1375-1382, 2005
457. Ferlicot S, Durrbach A, Ba N, Desvaux D, Bedossa P, Paradis V: The role of replicative senescence in chronic allograft nephropathy. Hum Pathol 34:924-928, 2003
458. Joosten SA, van H, V, Nolan CE, Borrias MC, Jardine AG, Shiels PG, van Kooten C, Paul LC: Telomere shortening and cellular senescence in a model of chronic renal allograft rejection. Am J Pathol 162:1305-1312, 2003
459. Schmid H, Henger A, Kretzler M: Molecular approaches to chronic kidney disease. Curr Opin Nephrol Hypertens 15:123-129, 2006
460. Eikmans M, Baelde HJ, De Heer E, Bruijn JA: RNA expression profiling as prognostic tool in renal patients: toward nephrogenomics. Kidney Int 62:1125-1135, 2002
461. Kretzler M, Cohen CD, Doran P, Henger A, Madden S, Grone EF, Nelson PJ, Schlondorff D, Grone HJ: Repuncturing the renal biopsy: strategies for molecular diagnosis in nephrology. J Am Soc Nephrol 13:1961-1972, 2002
462. Eikmans M, Ijpelaar DH, Baelde HJ, De Heer E, Bruijn JA: The use of extracellular matrix probes and extracellular matrix-related probes for assessing diagnosis and prognosis in renal diseases. Curr Opin Nephrol Hypertens 13:641-647, 2004
463. Mannon RB, Kirk AD: Beyond Histology: Novel Tools to Diagnose Allograft Dysfunction. Clin J Am Soc Nephrol 1:358-366, 2006
464. Strom TB, Suthanthiran M: Transcriptional profiling to assess the clinical status of kidney transplants. Nat Clin Pract Nephrol 2:116-117, 2006
465. Nicholson ML, Bailey E, Williams S, Harris KP, Furness PN: Computerized histomorphometric assessment of protocol renal transplant biopsy specimens for surrogate markers of chronic rejection. Transplantation 68:236-241, 1999
466. Diaz Encarnacion MM, Griffin MD, Slezak JM, Bergstralh EJ, Stegall MD, Velosa JA, Grande JP: Correlation of quantitative digital image analysis with the glomerular filtration rate in chronic allograft nephropathy. Am J Transplant 4:248-256, 2004
467. Pape L, Henne T, Offner G, Strehlau J, Ehrich JH, Mengel M, Grimm PC: Computer-assisted quantification of fibrosis in chronic allograft nephropaty by picosirius red-staining: a new tool for predicting long-term graft function. Transplantation 76:955-958, 2003
468. Grimm PC, Nickerson P, Gough J, McKenna R, Stern E, Jeffery J, Rush DN: Computerized image analysis of Sirius Red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function. J Am Soc Nephrol 14:1662-1668, 2003
469. Morel-Maroger SL, Killen PD, Chi E, Striker GE: The composition of glomerulosclerosis. I. Studies in focal sclerosis, crescentic glomerulonephritis, and membranoproliferative glomerulonephritis. Lab Invest 51:181-192, 1984
470. Furness PN: Extracellular matrix and the kidney. J Clin Pathol 49:355-359, 1996
471. Abrass CK, Berfield AK, Stehman-Breen C, Alpers CE, Davis CL: Unique changes in interstitial extracellular matrix composition are associated with rejection and cyclosporine toxicity in human renal allograft biopsies. Am J Kidney Dis 33:11-20, 1999
472. Strom TB: Rejection--more than the eye can see. N Engl J Med 353:2394-2396, 2005
473. Doi T, Striker LJ, Kimata K, Peten EP, Yamada Y, Striker GE: Glomerulosclerosis in mice transgenic for growth hormone. Increased mesangial extracellular matrix is correlated with kidney mRNA levels. J Exp Med 173:1287-1290, 1991
474. Peten EP, Striker LJ, Garcia-Perez A, Striker GE: Studies by competitive PCR of glomerulosclerosis in growth hormone transgenic mice. Kidney Int Suppl 39:S55-S58, 1993
475. Bergijk EC, Baelde HJ, De Heer E, Killen PD, Bruijn JA: Role of the extracellular matrix in the development of glomerulosclerosis in experimental chronic serum sickness. Exp Nephrol 3:338-347, 1995



476. Striker LJ: Modern renal biopsy interpretation: can we predict glomerulosclerosis? Semin Nephrol 13:508-515, 1993
477. Lipman ML, Stevens AC, Bleackley RC, Helderman JH, McCune TR, Harmon WE, Shapiro ME, Rosen S, Strom TB: The strong correlation of cytotoxic T lymphocyte-specific serine protease gene transcripts with renal allograft rejection. Transplantation 53:73-79, 1992
478. Strehlau J, Pavlakis M, Lipman M, Shapiro M, Vasconcellos L, Harmon W, Strom TB: Quantitative detection of immune activation transcripts as a diagnostic tool in kidney transplantation. Proc Natl Acad Sci U S A 94:695-700, 1997
479. Suthanthiran M: Molecular analyses of human renal allografts: differential intragraft gene expression during rejection. Kidney Int Suppl 58:S15-S21, 1997
480. Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M: Non-invasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 344:947-954, 2001
481. Muthukumar T, Dadhania D, Ding R, Snopkowski C, Naqvi R, Lee JB, Hartono C, Li B, Sharma VK, Seshan SV, Kapur S, Hancock WW, Schwartz JE, Suthanthiran M: Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med 353:2342-2351, 2005
482. Kamoun M, Boyd JC: Urinary FOXP3 messenger RNA and renal-allograft rejection. N Engl J Med 354:2291-2293, 2006
483. Soulillou JP: Immune monitoring for rejection of kidney transplants. N Engl J Med 344:1006-1007, 2001
484. Sharma VK, Bologa RM, Xu GP, Li B, Mouradian J, Wang J, Serur D, Rao V, Suthanthiran M: Intragraft TGF-beta 1 mRNA: a correlate of interstitial fibrosis and chronic allograft nephropathy. Kidney Int 49:1297-1303, 1996
485. Mas VR, Maluf DG, Archer KJ, Yanek K, King A, Cotterell A, Ferreira-Gonzalez A, Rodgers C, Fisher RA, Posner M: Study of mRNA growth factors in urinary cells of kidney transplant recipients as predictors of chronic allograft nephropathy. Transplantation 80:1686-1691, 2005
486. Eikmans M, Sijpkens YW, Baelde HJ, De Heer E, Paul LC, Bruijn JA: High transforming growth factor-beta and extracellular matrix mRNA response in renal allografts during early acute rejection is associated with absence of chronic rejection. Transplantation 73:573-579, 2002
487. Scherer A, Krause A, Walker JR, Korn A, Niese D, Raulf F: Early prognosis of the development of renal chronic allograft rejection by gene expression profiling of human protocol biopsies. Transplantation 75:1323-1330, 2003
488. Delarue F, Hertig A, Alberti C, Vigneau C, Ammor M, Berrou J, Akposso K, Peraldi MN, Rondeau E, Sraer JD: Prognostic value of plasminogen activator inhibitor type 1 mRNA in microdissected glomeruli from transplanted kidneys. Transplantation 72:1256-1261, 2001
489. Revelo MP, Federspiel C, Helderman H, Fogo AB: Chronic allograft nephropathy: expression and localization of PAI-1 and PPAR-gamma. Nephrol Dial Transplant 20:2812-2819, 2005
490. Nicholson ML, Waller JR, Bicknell GR: Renal transplant fibrosis correlates with intragraft expression of tissue inhibitor of metalloproteinase messenger RNA. Br J Surg 89:933-937, 2002
491. Seiler M, Brabcova I, Viklicky O, Hribova P, Rosenberger C, Pratschke J, Lodererova A, Matz M, Schonemann C, Reinke P, Volk HD, Kotsch K: Heightened expression of the cytotoxicity receptor NKG2D correlates with acute and chronic nephropathy after kidney transplantation. Am J Transplant 7:423-433, 2007
492. Nocera A, Tagliamacco A, Ferrante A, Fontana I, Rolla D, De Palma R, Del Galdo F, Ginevri F, Barocci S, Valente U: Cytotoxic molecule mRNA expression in chronically rejected human kidney allografts. Transplant Proc 37:2476-2478, 2005
493. Kirk AD, Jacobson LM, Heisey DM, Radke NF, Pirsch JD, Sollinger HW: Clinically stable human renal allografts contain histological and RNA-based findings that correlate with deteriorating graft function. Transplantation 68:1578-1582, 1999
494. Roos-van Groningen MC, Scholten EM, Lelieveld PM, Rowshani AT, Baelde HJ, Bajema IM, Florquin S, Bemelman FJ, De Heer E, De Fijter JW, Bruijn JA, Eikmans M: Molecular comparison of calcineurin inhibitor-induced fibrogenic responses in protocol renal transplant biopsies. J Am Soc Nephrol 17:881-888, 2006
495. Sarwal MM: Chipping into the human genome: novel insights for transplantation. Immunol Rev 210:138-155, 2006
496. Hotchkiss H, Chu TT, Hancock WW, Schroppel B, Kretzler M, Schmid H, Liu Y, Dikman S, Akalin E: Differential expression of profibrotic and growth factors in chronic allograft nephropathy. Transplantation 81:342-349, 2006
497. Donauer J, Rumberger B, Klein M, Faller D, Wilpert J, Sparna T, Schieren G, Rohrbach R, Dern P, Timmer J, Pisarski P, Kirste G, Walz G: Expression profiling on chronically rejected transplant kidneys. Transplantation 76:539-547, 2003
498. Eikmans M, Roos-van Groningen MC, Sijpkens YW, Ehrchen J, Roth J, Baelde HJ, Bajema IM, De Fijter JW, De Heer E, Bruijn JA: Expression of surfactant protein-C, S100A8, S100A9, and B cell markers in renal allografts: investigation of the prognostic value. J Am Soc Nephrol 16:3771-3786, 2005
499. O'Riordan E, Orlova TN, Mei JJ, Butt K, Chander PM, Rahman S, Mya M, Hu R, Momin J, Eng EW, Hampel DJ, Hartman B, Kretzler M, Delaney V, Goligorsky MS: Bioinformatic analysis of the urine proteome of acute allograft rejection. J Am Soc Nephrol 15:3240-3248, 2004
500. Schaub S, Rush D, Wilkins J, Gibson IW, Weiler T, Sangster K, Nicolle L, Karpinski M, Jeffery J, Nickerson P: Proteomic-based detection of urine proteins associated with acute renal allograft rejection. J Am Soc Nephrol 15:219-227, 2004
501. Wishart DS: Metabolomics: a complementary tool in renal transplantation. Contrib Nephrol 160:76-87, 2008
502. Kim JM, Wu H, Green G, Winkler CA, Kopp JB, Miner JH, Unanue ER, Shaw AS: CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. Science 300:1298-1300, 2003
503. Hinkes B, Wiggins RC, Gbadegesin R, Vlangos CN, Seelow D, Nurnberg G, Garg P, Verma R, Chaib H, Hoskins BE, Ashraf S, Becker C, Hennies HC, Goyal M, Wharram BL, Schachter AD, Mudumana S, Drummond I, Kerjaschki D, Waldherr R, Dietrich A, Ozaltin F, Bakkaloglu A, Cleper R, Basel-Vanagaite L, Pohl M, Griebel M, Tsygin AN, Soylu A, Muller D, Sorli CS, Bunney TD, Katan M, Liu J, Attanasio M, O'toole JF, Hasselbacher K, Mucha B, Otto EA, Airik R, Kispert A, Kelley

GG, Smrcka AV, Gudermann T, Holzman LB, Nurnberg P, Hildebrandt F: Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. Nat Genet 38:1397-1405, 2006
504. Niaudet P: Genetic forms of nephrotic syndrome. Pediatric Nephrology V19:1313-1318, 2004
505. Kriz W: TRPC6 - a new podocyte gene involved in focal segmental glomerulosclerosis. Trends in Molecular Medicine 11:527-530, 2005
506. Endlich K, Kriz W, Witzgall R: Update in podocyte biology. Curr Opin Nephrol Hypertens 10:331-340, 2001