On the pathology of focal segmental glomerulosclerosis

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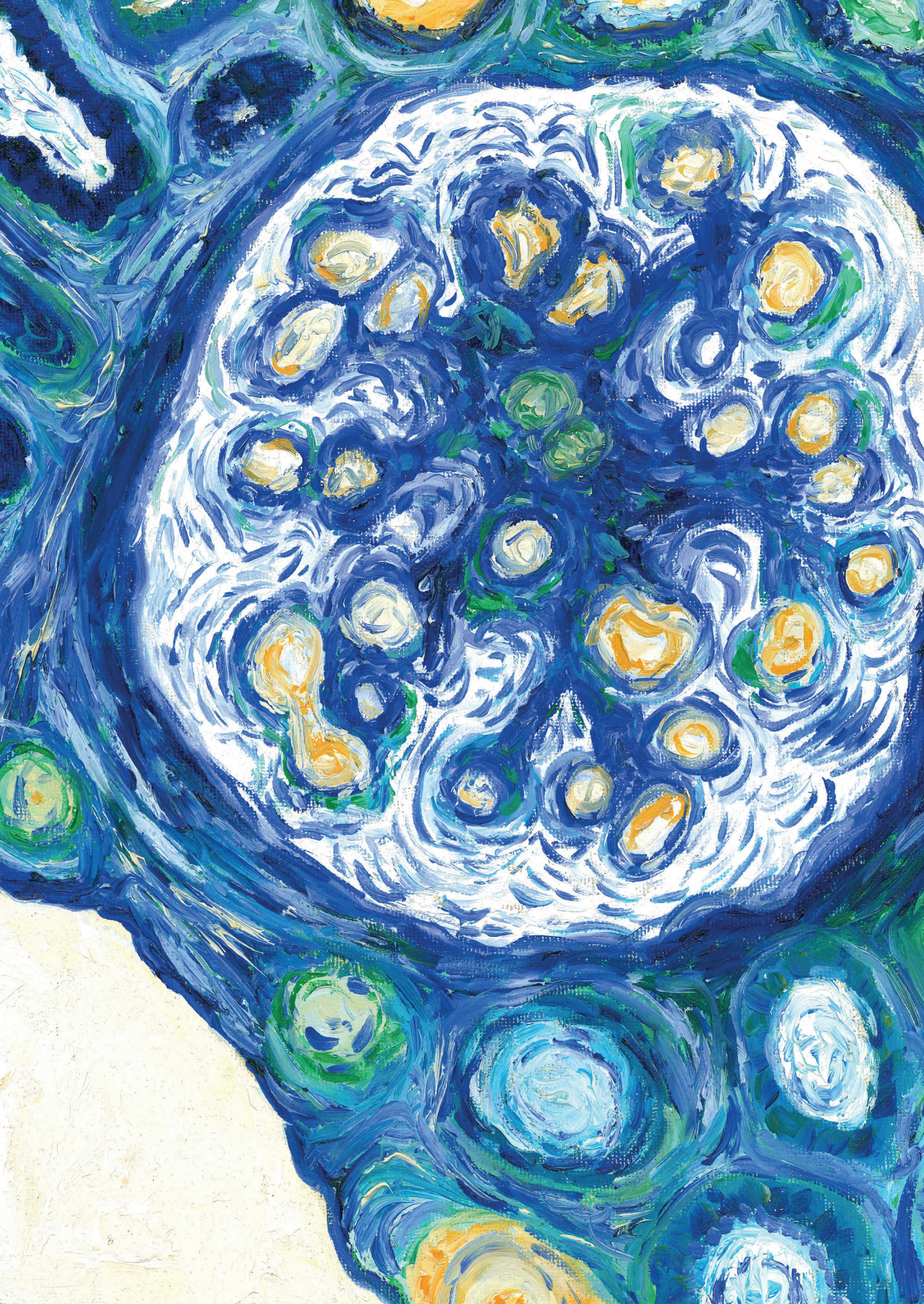

1 Introduction

Prologue: glomerulogenesis

Glomerulogenesis is a play in two acts: the first is the formation of tubulocystic structures through interaction of the metanephric mesenchyme and the uretic bud.[1,2] The second relates to the capillary invasion of these early glomerular structures. The complex interaction between cells from metanephric mesenchymal lineages and cells from angioblast lineages gives rise to the unique hybrid structure that is the glomerulus.[3]

Before capillary invasion, mesenchymal cells transform into an epithelial vesicle aptly called the comma-shaped body.[1] In this comma-shaped body, parietal epithelial cells and premature podocytes reside. Together, they form the urinary space. These premature podocytes are not yet fully differentiated, not having the typical podocyte structural elements as interdigitating foot processes and slit diaphragms.[1,3,4] Following the formation of the comma-shaped body, a vascular cleft is formed which results in the so called S-shaped body. It is in this cleft where vascular invasion occurs. In response to signaling molecules produced by the premature podocytes, angioblasts with the capacity to differentiate into endothelial cells migrate to the vascular cleft. Here, they subsequently differentiate into glomerular endothelial cells.[1,3] The critical role for podocyte-endothelial signaling in the formation of the glomerulus has been shown in models of podocyte-specific knockdown of vascular endothelial growth factor A, a key regulator of podocyte-endothelial interaction.[5] Impaired podocyte-endothelial signaling in these animals results in inadequate glomerular vascularization. Another critical step in glomerular vascularization is the recruitment of mesangial cells. This cell population arises when the endothelial precursor cells start to secrete platelet-derived growth factor. Within the premature glomerulus, mesangial cells attach to the vasculature and pull at the endothelial cells, in this way displaying vascular smooth muscle cell-like properties. The subsequent invagination of the capillaries gives rise to the unique segmented structure of the glomerular capillary loop.[6]

After initial vascular invasion, endothelial-podocyte signaling continues and both cell types undergo synergistic differentiation. During this mutual differentiation process, the glomerular endothelial cells and podocytes develop their characteristic phenotypic features: endothelial fenestrae and podocyte interdigitating foot processes. The differentiating podocytes and endothelial cells both continue to produce a basement membrane. This double layered basement membrane fuses into one shared glomerular basement membrane lined by endothelial cells on the inside and podocytes on the outside: the glomerular filtration barrier is born.[7]

The evolution of the glomerulus has led to an extraordinary and highly specialized structure that deals with two contradicting principles: filtering the blood of wasteful products while maintaining the blood's vital components. However, its unique properties and structure also make this important micro-organ prone to injury and disease. A notorious clinicopathological entity that affects the glomerulus is focal segmental glomerulosclerosis, which is characterized by podocyte injury and segmental lesions of the glomerular tuft. The work described in this thesis evaluates and investigates the mechanisms that contribute to glomerular malfunction in this clinicopathological entity.

Part 1: the mature glomerular filtration barrier

The term glomerular filtration barrier (GFB) was coined in the 1950s when electron microscopy made it possible to observe its independent structures.[8] The GFB consists of three layers: the fenestrated glomerular endothelium, the podocytes with their foot processes and in between their shared glomerular basement membrane (GBM) (Figure 1). The GFB is a permselective barrier that makes filtration of blood plasma possible while keeping important macromolecules in the circulation. It is highly selective, as it is freely permeable to water and small molecules, whereas only 0.008% of plasma albumin passes through the GFB.[9] Each single layer is essential for proper filter function of the GFB. The morphology, components and the role in maintaining permselectivity of each individual layer will be discussed below.

The glomerular endothelium

The glomerular endothelium consists of uniquely differentiated endothelial cells. They are characterized by the presence of transcellular fenestrations of approximately 70 nm in diameter. These 'holes' occupy 30-40% of the cell surface and facilitate the high water permeability.[10] Initially it was thought that the only barrier function of the glomerular endothelium was to exclude cellular components from passing the GFB. However, recently it has become evident that the endothelium also plays a role in handling of albumin and other macromolecules. This notion first came to life when attention was focused on the endothelial glycocalyx, a layer of glycosaminoglycans covering the entire endothelial surface, including the fenestrations. The glycocalyx is a complex meshwork of proteoglycans and glycoproteins anchored to the cell surface. In addition, secreted molecules as hyaluronan and circulating molecules are trapped in the glycocalyx.[11,12] The anchored and loosely adherent molecules together form the endothelial surface layer. Several studies have shown that the endothelial surface layer is reduced in diabetic nephropathy, which causes proteinuria.[13,14] Additionally, animal models for glomerulosclerosis showed that

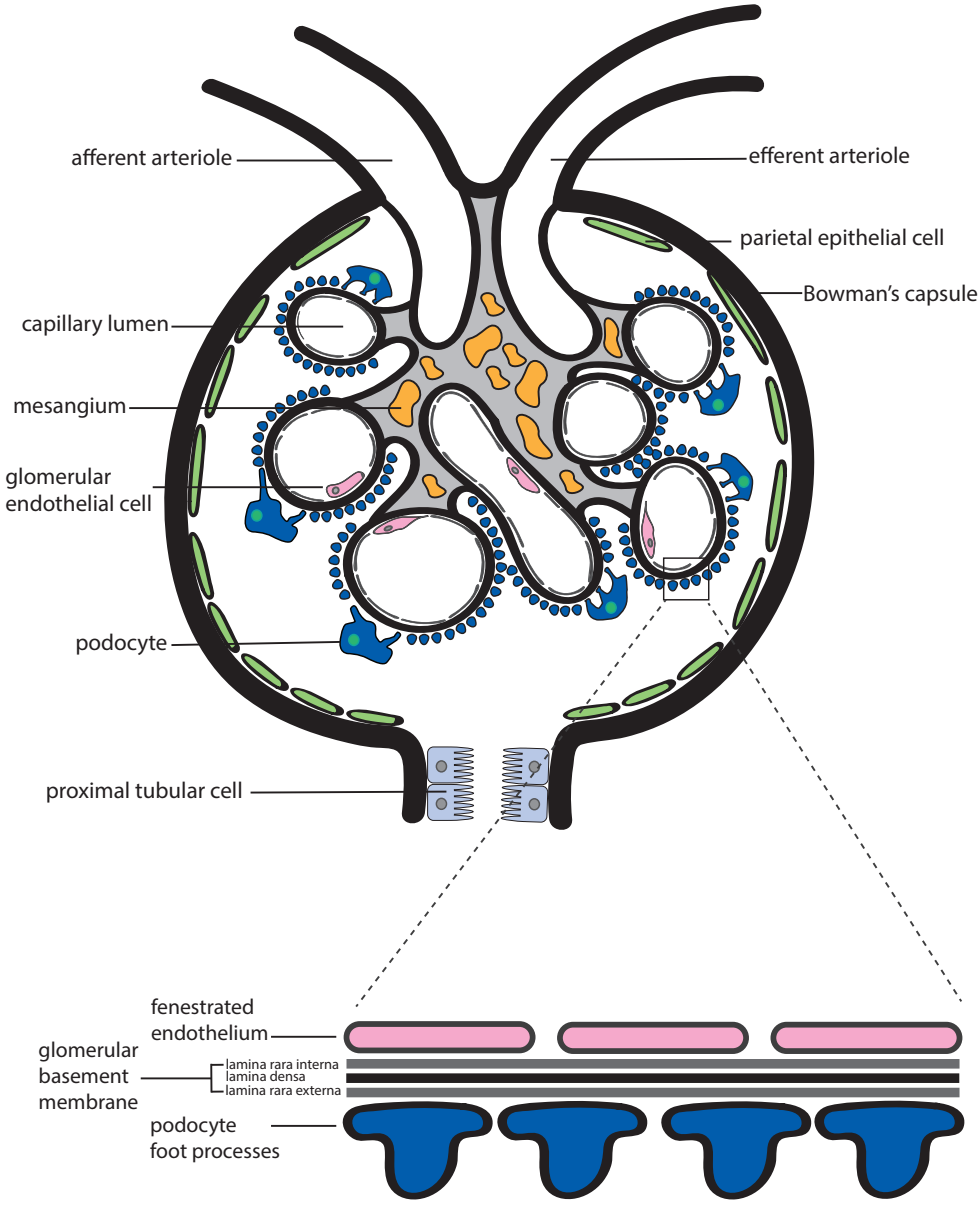

Figure 1. Schematic depiction of a renal glomerulus and the glomerular filtration barrier (enlarged in inset).

the endothelial surface layer is the first GFB component to be affected in the development of albuminuria.[15,16] It is now clear that the fenestrated glomerular endothelium is not only a cellular sieve, but is actively engaged in maintaining the barrier function of the GFB.

The glomerular basement membrane

The GBM is formed by both endothelial cells and podocytes. Its main components are type IV collagens with α 3, 4 and 5 side chains and laminin-521. Negatively charged heparan-sulfate proteoglycans form another major component of the GBM.[17] The GBM has three main functions, all contributing to the permselectivity of the GFB: 1) it provides structural support to both endothelial cells and podocytes, 2) it plays an important role in cell-cell and cell-matrix crosstalk and 3) it acts as a size-selective gel for diffusion of molecules passing the GFB.[17] The second is a current topic of research and increasing evidence suggests that impaired signaling from podocytes to the GBM is an important mediator of proteinuria.[18-20] The concept of the GBM as a size-selective gel rather than a sieve was proposed by Oliver Smithies, who showed that molecules in the GBM diffuse like they would in a gel-like structure.[21,22] These studies also show that the majority of serum macromolecules and charged molecules is being retained by the GBM, never reaching the podocyte slit pore.[23] Reducing the percentage of the major components of the GBM (laminin or collagen) leads to the development of nephrotic syndrome.[17] Gradually, the GBM is thumping the podocyte as the major direct barrier to albumin and other macromolecules of the GFB.

The podocyte

The podocyte is the last barrier that molecules face when passing the GFB. The podocyte is a highly specialized epithelial cell with a distinct morphology. During glomerulogenesis, podocytes lose their basolateral attachments and spread out over the surface of the GBM. During this spreading they form primary and secondary processes that interdigitate with processes from adjacent podocytes, forming a vast network of cell-cell interactions.[4] In between these secondary foot processes lies a structure called the slit pore, which plays a crucial role in maintaining glomerular permselectivity. The first slit pore component was identified in 1998 when researchers identified mutations in the gene *NPHS1*, encoding the protein nephrin, as the cause of the congenital nephrotic syndrome of the Finish type.[24] In the years that followed, many more components of the slit pore were discovered. Mutations in these slit pore associated genes account for a large number of the genetic causes of nephrotic syndrome and proteinuria.[25] Foot process effacement – a distinct morphological alteration of these slit pores and secondary foot processes – can be observed in virtually all diseases characterized by proteinuria. The strong

associations between slit pore alterations and the development of proteinuria have led to the proposal that the slit pore forms the main barrier to albumin and other macromolecules. However, recent data suggest that the slit pore itself might not have direct size-selective sieving properties. Its role in maintaining the GFB would rather be established by its complex signaling functions, regulating both extracellular and intracellular signaling pathways. It is proposed that proteinuria, caused by mutations or acquired defects of the slit pore, is a result of impaired signaling of the podocyte to the endothelial cells and the GBM.[17,19] It is clear that podocyte and slit pore integrity are indispensable – either direct or indirect – for the permselective properties of the GFB.

Slit pore and podocyte cytoskeleton signaling

The slit pore is a highly specialized cell-cell junction that is anchored to the actin cytoskeleton of the secondary foot processes of the podocytes via a series of transmembrane proteins. The slit pore consists of an extracellular, a transmembrane and an intracellular compartment (Figure 2). The three compartments are connected via an ingenious system of molecules, each with its own specialized function. The extracellular compartment of the slit pore consists primarily of the extracellular tails of the neural junction proteins nephrin and nehp1.[25] Nephrin is the only molecule that spans the gap between two adjacent foot processes, thereby forming a zipper-like structure within the slit pore.[26] The transmembrane portion of the slit pore consists of a variety of molecules including integral membrane proteins, ion channels such as 'transient receptor potential cation channel subfamily C member 6' (TRPC6) and molecules spanning the extra- and intracellular compartment (e.g. nephrin). The intracellular compartment of the slit pore forms the connection between the slit pore and the podocyte cytoskeleton. The cytoplasmic tail of nephrin, podocin and CD2-associated protein are important components of the intracellular compartment.[25,27]

The slit pore is crucial for maintaining permselectivity of the GFB and genetic studies have shown that mutations in a single slit pore molecule can lead to proteinuria and loss of podocyte integrity.[24,28-30] Although the slit pore was originally thought of as a molecular sieve, new research has brought to light that the slit pore is actually an important signal transduction hub. Signal transduction through the slit pore can be subdivided into three categories: detection of incoming signals, the signal-transducing machinery and the downstream signaling effectors.[25] Podocytes are exposed to a variety of incoming signals, including physical stress generated by constantly oscillating mechanical pressure, the challenges of the extremely polarized cellular architecture necessitating the maintenance of a vastly enlarged apical membrane and the exposure to paracrine and autocrine cytokines. How

podocytes detect these incoming signals is incompletely understood, but advances have recently been made in this field. One possible mechanosensor might be the slit pore molecule podocin, whose molecular structure shows similarities with mechanosensing molecules in neurons.[25,31] Another slit pore molecule with mechanosensing properties is the ion channel TRPC6, which displays increased activity in response to membrane stretch. Interestingly, several studies have shown that podocin interacts with TRPC6 upon mechanical stress and differentially modulates its activity.[31-33] Following incoming signals, signal transduction through the slit pore to the podocyte cytoskeleton is of major importance to maintain podocyte health. Many studies have proposed nephrin as the core biomechanical component of the slit pore that is crucial for signal transduction. Without nephrin, formation of the slit pore does not even occur.[34,35] In line with this, children lacking nephrin present with nephrotic syndrome form birth. Interestingly, nephrin mutations of the FINminor type lead to a similar phenotype as total loss of nephrin, while the FINminor mutation only causes a truncation of the cytoplasmic tail.[24] This observation led to the discovery that phosphorylation of the intracellular tail of nephrin is the key between signaling from the slit pore to the actin cytoskeleton and is critical for stabilizing the podocyte cytoarchitecture.[36] Another example of the importance of the interaction of nephrin with downstream effector molecules is that of the 'nephrin/NCK1/NCK2/N-WASP' pathway, which enables accurate actin polymerization and nucleation.[25,37] Lastly, slit pore signaling seems to play an important role in maintaining the unique apically oriented structure of the podocyte. Various polarity molecules anchor to the slit pore and form a signaling network that distinguishes the apical from the basolateral compartments of the podocyte.[25] In conclusion, the slit pore, originally thought of as a molecular sieve, is closely associated with many aspects of podocyte function, and thereby a crucial component of the GFB.

From the slit pore we continue to the primary and secondary processes of the podocyte, which also convey unique structural elements. The primary processes mainly consist of microtubules and intermediate filaments. The secondary processes are composed of two different types of actin networks: the central bundle and the cortical bundle.[38] The filaments in these bundles mainly consist of F-type actin and are crosslinked by α-actinin-4 molecules. During podocyte foot process effacement the cortical and central bundle rearrange into a single, dense actin mat at the apical site.[39] These cytoskeletal rearrangements are accompanied by an increased expression of actin and α-actinin-4, with a clear localization of α-actinin-4 to dense areas. Actin cytoskeleton signaling is necessary for the formation of stress fibres under stressful conditions such as mechanical stretch. Impaired signaling leads to a more brittle podocyte cytoskeleton that is less resistant to injury.[40] Mutations

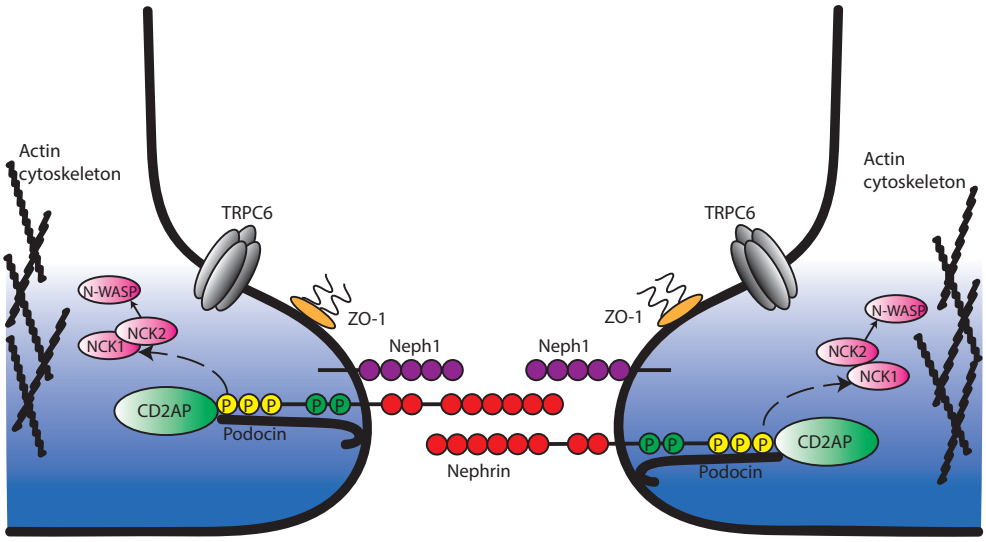

Figure 2. Schematic view of the podocyte slit pore.

in *ACTN4*, the gene encoding α-actinin-4, lead to impaired cytoskeletal dynamics due to decreased protein stability. This illustrates its importance in maintaining a healthy podocyte cytoskeleton.[19] Synaptopodin, an actin-binding protein, interacts with α-actinin-4. Although loss of synaptopodin does not lead to alterations in the ultrastructural morphology of the podocyte, synapotopodin deficient mice are less resistant to stress and show impaired recovery after toxic podocyte injury.[19,41,42]
Finally, a key determinant of podocyte and GFB function is podocyte adhesion to the GFB. Podocyte loss is a final common pathway in many glomerular diseases and can be the result of failure of the podocyte to adhere to the GBM. Podocytes are anchored to the GBM via the adhesome. The key structural components of the adhesome are integrins and integrin-associated linker molecules, which form complexes known as focal adhesions.[19] Although podocyte detachment from the GBM poses a major problem in several glomerular diseases, much about how these structures are anchored to each other is still unknown.

Part 2: focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) is a histopathological entity of glomerular injury characterized by segmental sclerosis of the glomerular tuft. FSGS is thought to be the result of various types of podocyte injury, leading to reduced permselectivity of the GFB and to proteinuria. Cases with FSGS are divided in primary and secondary FSGS. The definitions of these two are not always straightforward. Originally, the term primary FSGS (also known as idiopathic FSGS) was coined to differentiate FSGS with no known cause from secondary FSGS, which is caused by known pathogenic events. Yet, primary FSGS often refers to a disease entity characterized by primary changes to the podocyte and nephrotic syndrome. Secondary FSGS refers to the secondary development of FSGS lesions in response to many other renal diseases or to another known pathological event, such as viral infections or drug toxicity. Occasionally, secondary FSGS is also referred to as adaptive FSGS, a term that is mainly based on the secondary development of FSGS due to glomerular hyperfiltration. How to fit the later discovered genetic forms of FSGS into this system of primary and secondary FSGS is still a subject of debate. It has been proposed to categorize genetic forms as primary FSGS, since they develop due to primary podocyte injury and the clinical presentation is often similar to what is usually seen in primary FSGS. However, the genetic forms are generally categorized as secondary FSGS, because the cause of podocyte injury is known. Lastly, it has also been proposed to view genetic FSGS as a separate group.

In spite of these differences in nomenclature, most cases can quite clearly be categorized as either primary, secondary or genetic. All in all, it is important to acknowledge that FSGS is a pattern of glomerular injury that arises due to various types of podocyte injury. In the following section, the histological, clinical and demographical characteristics of FSGS will be discussed, as well as the etiology, pathogenesis and treatment options.

History and histopathology of the lesion

The term FSGS evolved from early studies conducted in the nineteen-twenties investigating the light microscopic findings in kidneys of children with nephrotic syndrome. The first histopathological description of the disease actually focused on the tubular changes rather than the glomerular changes: typical vacuolization and microscopic lipid droplets in proximal tubules led to the term ‘lipoid nephrosis’.[43] A few years later, Fahr was the first to describe that patients with persistent lipoid nephrosis displayed focal glomerular lesions.[44] The subsequent study by Rich, who conducted a detailed analysis of autopsy material of patients who died from the nephrotic syndrome, is considered the first true description of what we now call

focal segmental glomerulosclerosis with hyalinosis (e.g. FSGS).[45] The focal and segmental character of FSGS was already recognized in this study: the obliterative lesions initially only affected a minority of the glomeruli (focal) and only a portion of the glomerular tuft (segmental). The study by Rich provoked a re-examination of the pathology underlying the idiopathic nephrotic syndrome. In 1970, Churg *et al.* discovered the association between FSGS and steroid-resistant nephrotic syndrome, thereby introducing FSGS as a distinct pathological entity in the spectrum of the nephrotic syndrome.[46] Already in this study, the morphological heterogeneity of FSGS was recognized. With the emergence of the renal biopsy, renal pathology flourished and more detailed descriptions of FSGS and its various variants emerged. Over the years, several distinct variants of FSGS were recognized, which finally led to the histological classification of FSGS in 2004: the Columbia classification.[47] The Columbia classification distinguishes five morphological patterns of FSGS lesions: the glomerular tip lesion, cellular FSGS, collapsing FSGS, perihilar FSGS and FSGS not otherwise specified (NOS) (Figure 3 and Table 1). Since several of the distinct lesions can be present in a single biopsy, the authors chose a hierarchical system of exclusion for the classification. Thus, even when more than one of the distinct histological lesions are present in a biopsy, it can only be assigned to one of the five categories (Table 1).

The glomerular tip lesion was the first variant described by Howie and Brewer in 1984.[48] They characterized the tip lesion as a rather small segmental lesion at the tubular pole of the glomerulus. The current classification defines glomerular tip lesions as segmental lesions involving the tip domain (the outer 25% of the tuft next to the origin of the proximal tubule) of the glomerulus, with either an adhesion between the tuft and the Bowman's capsule at the tubular lumen or neck, or confluence of podocytes with parietal or tubular epithelial cells at the tubular lumen or neck. The segmental tip lesion can either be characterized by sclerosis or hypercellularity in the form of foam cells.[47] Thus, sclerosis is not a necessary component for the diagnosis of the FSGS tip variant. The FSGS tip variant generally presents with nephrotic syndrome with abrupt onset. Its response rate to corticosteroids is high and it is generally considered to have a favorable prognosis.[49]

The cellular FSGS lesion was first mentioned in a paper by Schwartz and Lewis in 1985.[50] The lesions they described comprised both extra and endocapillary hypercellularity. In hindsight, the extracapillary hypercellularity consisting of podocyte proliferation or hypertrophy that these authors coined as cellular FSGS, more accurately described the lesion that would later be called collapsing FSGS. The current classification defines cellular FSGS as endocapillary hypercellularity involving at least 25% of the tuft and causing occlusion of the capillary lumen. Endocapillary

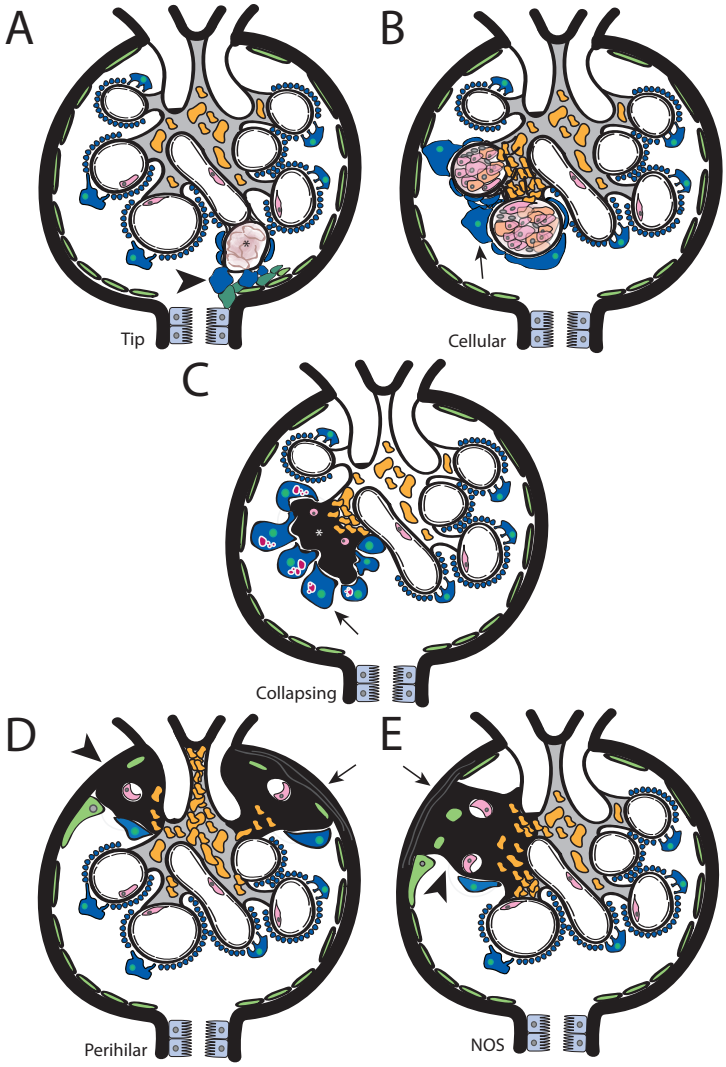

Figure 3. Schematic depiction of the five histological variants of focal segmental glomerulosclerosis (FSGS) according to the Columbia classification.

(A) FSGS tip lesion (arrowhead) showing podocyte and parietal epithelial cell hypertrophy at the tubular pole of the Bowman's capsule. The asterisk indicates foamy changes in the capillary lumen. (B) Cellular variant of FSGS. Capillary lumina are occluded with cellular components. Moreover, podocytes covering these capillary loops show morphological changes with foot process effacement (arrow). (C) Collapsing variant of FSGS. A segment of the glomerular tuft shows collapse of the capillary loops (asterisk) with glomerular basement membrane wrinkling. In addition, podocyte hypertrophy and hyperplasia can be observed (arrow). Affected podocytes may typically show vacuolization. (D) Perihilar variant of FSGS. Segmental increase in matrix located at the glomerular hilus (arrowhead). (E) FSGS not otherwise specified (NOS). Segmental increase in matrix occluding capillary lumine. Splitting of the Bowman's capsule can be observed (arrow in D and E).

Table 1. The Columbia classification

Histological variants of focal segmental glomerulosclerosis

Variant	Description	Exclusion criteria
Collapsing variant	At least 1 glomerulus with segmental or global collapse and overlying podocyte hypertrophy and hyperplasia.	None
Tip variant	At least 1 segmental lesion involving the tip domain (outer 25% of tuft next to origin of proximal tubule). The tubular pole must be identified in the defining lesion. The lesion must have either an adhesion or confluence of podocytes with parietal or tubular cells at the tubular lumen or neck. The lesion may be cellular or sclerosing.	Presence of collapsing variant
Cellular variant	At least 1 glomerulus with segmental endocapillary, hypercellularity occluding lumina, with or without foam cells and karyorrhexis.	Presence of tip and/or collapsing variant
Perihilar variant	At least 1 glomerulus with perihilar hyalinosis, with or without sclerosis. >50% of glomeruli with segmental lesions must have perihilar sclerosis and/or hyalinosis.	Presence of cellular, tip, and/or collapsing variant
Not otherwise specified (NOS)	At least 1 glomerulus with segmental increase in matrix obliterating the capillary lumina. There may be segmental glomerular capillary wall, collapse without overlying podocyte hyperplasia.	Presence of perihilar, cellular, tip, and/or collapsing variant

cells typically include foam cells, macrophages, leukocytes, lymphocytes or endothelial cells. Neither hyalinosis nor segmental sclerosis are required features.[47]

Although lesions with collapse as a characteristic feature may have been observed in earlier publications, the term 'collapsing FSGS' was first coined by Weiss *et al.* in 1986.[51] In a subgroup of FSGS patients with severe nephrotic syndrome and rapid progression to renal failure, the authors described a lesion that was defined by both collapse of the glomerular tuft and epithelial hypercellularity. Soon after this publication, collapsing FSGS was found to be one of the key characteristics of HIV-associated nephropathy.[52,53] The current classification describes collapsing FSGS (also known as collapsing glomerulopathy) as segmental or global collapse of the tuft with podocyte hypertrophy and hyperplasia.[47] Several studies have indicated that the hyperplastic cells in collapsing lesions can also be of parietal epithelial origin.[54,55] The prominent podocytes often contain intracytoplasmic protein droplets. The presence of a collapsing lesion pre-empts all other variants of FSGS.[47] Collapsing FSGS is generally believed to have the worst prognosis compared to other variants of FSGS.[49] The variant is often described in association with viral infections and drug toxicity, although it is not uncommon in primary cases of FSGS as well.[56]

The perihilar variant of FSGS shows many similarities with the NOS variant. That the perihilar location could be a distinctive feature of FSGS lesions, was not initially recognized. After several studies showed clinical correlations between the location of the segmental sclerotic lesion[57,58] and a close association of the perihilar location and glomerular hyperfiltration as a cause of FSGS,[59-61] the perihilar location in combination with hyalinosis was eventually considered a separate variant. The current classification defines perihilar FSGS as at least one glomerulus with perihilar hyalinosis, with or without sclerosis. More than 50% of the glomeruli with FSGS must contain perihilar sclerosis. This variant is usually accompanied by sub-nephrotic proteinuria and is associated with adaptive forms of FSGS caused by glomerular hyperfiltration.[47]

The last category of the Columbia classification is the NOS variant of FSGS, which is the most common variant of FSGS in most studies. FSGS NOS can be viewed as FSGS that does not meet any of the defining features of the above variants. It is defined by focal and segmental consolidation of the tuft by increased extracellular matrix obliterating the capillary lumina. Collapsing, tip and cellular FSGS must be excluded and perihilar lesions must not be present in more than 50% of affected glomeruli in order to diagnose FSGS NOS.[47] Nonetheless, podocyte hypertrophy or hyperplasia may occur. Adhesions with Bowman's capsule are commonly observed as well.[49]

Clinical presentation

Patients with FSGS typically present with nephrotic syndrome. Especially primary FSGS is strongly associated with this clinical presentation. Nephrotic syndrome is classically characterized by four clinical features: nephrotic range proteinuria (>3.5g/day), hypoalbuminemia, edema and hyperlipidaemia. Only the first two are essential for the diagnosis of nephrotic syndrome, since not all patients with nephrotic syndrome present with edema and hyperlipidaemia.[62] The pathophysiological mechanism underlying nephrotic syndrome is increased permeability of the GFB, resulting in protein leakage from the blood into the urine. When the protein level in the urine reaches the critical amount >0.3g/day, this is considered proteinuria. A true nephrotic syndrome only develops in patients with heavy proteinuria (>3.5g/day). Hypoalbuminemia is the result of this heavy leak of protein and develops when the loss of albumin via the urine exceeds the ability of the liver to compensate for this loss. The reduction in serum albumin/serum proteins contributes to a reduction in serum colloid osmotic pressure. Due to the reduced colloid osmotic pressure, water flow from the interstitial space into the vessel lumen diminishes. The increased water volume in the interstitial space gives rise to the development of peripheral edema (underfill hypothesis).[63] Apart from reduced colloid oncotic pressure, a primary sodium retention directly induced by the underlying kidney disease also contributes to edema development (overfill hypothesis).[63] Typical locations for edema in cases of nephrotic syndrome are periorbital and tibial edema, but extreme forms of full body edema can develop as well.

There are various renal diseases which present with nephrotic syndrome and a renal biopsy can be helpful in determining the definitive diagnosis. An important differential diagnostic consideration of FSGS is minimal change disease (MCD), another podocytopathy with a clinical presentation that resembles that of FSGS. It is important to make the distinction between MCD and FSGS, because patients with MCD generally respond much better to treatment and therefore have a significantly better prognosis.[64,65] Several clinical features may be helpful to differentiate between MCD and FSGS. For instance, hypertension and loss of renal function are not uncommon in patients with FSGS, while they are rare in patients with MCD. However, the absence or presence of either one of these features are not confined to MCD or FSGS. Therefore, the golden standard for distinguishing FSGS from MCD is a renal biopsy. A biopsy of a patient with FSGS will show the characteristic glomerular lesions whereas renal biopsies from patients with MCD hardly show any light microscopic changes, hence the term “minimal change”. Podocyte lesions are of course encountered by electron microscopy but do not distinguish between FSGS and MCD. Due to the focal character of FSGS, there is always a possibility that FSGS could have been missed due to sampling error.

Epidemiology

FSGS is the most common cause of adult onset nephrotic syndrome accounting for 35% of all cases in a Caucasian population and up to 50% in African-Americans.[66] This translates into an absolute incidence of FSGS in cases of nephrotic syndrome of 1 per 100.000 person years.[67] In children, FSGS is the second most common cause of nephrotic syndrome and the most frequent cause of steroid-resistant nephrotic syndrome.[68-70] The incidence of FSGS lesions secondary to other renal diseases is 2.3 per 100.000 person years.[71] A critical note must be added to the reported incidence rates of FSGS. Absolute incidence and prevalence of FSGS are difficult to ascertain because of the large global variation in occurrence of FSGS, differences in biopsy policy over countries and hospitals, and changing definitions of FSGS over time. Nonetheless, several large studies show compelling evidence for an increase in the incidence of FSGS, absolute and relative to other glomerular diseases. Thus, the global burden of the disease is rising.[71-73]

Patients with FSGS have a relatively poor outcome compared to patients with other primary glomerular diseases. FSGS is the most common primary glomerular disease identified in patients with end-stage renal disease (ESRD), and patients with nephrotic syndrome due to FSGS are most likely to develop ESRD and will be in need of renal replacement therapy.[74] In approximately 30% of those patients that receive a kidney transplant due to primary FSGS, the disease recurs in the allograft kidney.[75,76] More information on the nature of recurrent FSGS will be provided in a later section.

Etiology

FSGS is a diverse histopathological entity that arises after podocyte injury of various causes. Some of these have been discovered, but for many patients with FSGS, especially primary FSGS, the cause of the disease remains unidentified. In fact, primary FSGS is for practical reasons often defined as the exclusion of any identifiable cause of FSGS. However, advances have been made in identifying pathological mechanisms leading to initial podocyte damage in this patient group. The phenomenon of rapid recurrence of FSGS after transplantation led to the belief that a systemic factor must be the cause of the disease, at least in a subset of patients. Plasmapheresis therapy is often effective in these patients, which suggests that the systemic pathological factor is a molecule that can be eliminated via plasmapheresis.[65,77] What this systemic factor could be, is currently a topic of investigation. The most intriguing notion is the presence of a circulating permeability factor as the cause of primary FSGS. The candidates for circulating factors include cardiotrophin-like cytokine factor 1, ApoA1b and the soluble urokinase–type plasminogen activator receptor (suPAR).[74] Other researchers have focused on the possibility that primary FSGS is, after all, an auto-immune mediated

disease. Delville *et al.* suggested that anti-CD40 antibodies could play an important role in the development of FSGS.[78] Yet, none of these (circulating) factors have been conclusively proven to be the actual causative factor of primary FSGS. It is very likely that, in the future, patients now diagnosed as having primary FSGS, will be reassigned to alternative etiologies, including new genes, environmental factors, and/or circulating factors.

Apart from a systemic factor, podocyte damage in FSGS can also arise due to intrinsic podocyte abnormalities. Various genetic mutations in podocyte-associated genes have been shown to lead to the development of FSGS. The most common mutations can be found in the genes encoding the slit pore proteins nephrin and podocin.[79] In addition, many other mutations have been recognized over the last few years, both in childhood and adult onset FSGS (listed in Table 2). Most of these mutations occur in podocyte-associated genes. However, some of them have been identified in other cells than podocytes and lead indirectly to podocyte damage. Other genes pose a higher risk of developing FSGS later in life. The most well-known is the *APOL1* genetic variant that occurs at a high frequency in populations of West African ancestry and partially explains the high incidence of FSGS in the African American population. It is speculated that a second hit might be necessary in patients with these genetic variants in order to develop FSGS and several associations have been found in the case of *APOL1*.[80] The most common genetic variants associated with FSGS can be found in Table 2.[74,81,82]

The development of secondary FSGS lesions can be the result of various types of renal injury. FSGS has been described as a secondary phenomenon in numerous primary glomerulopathies, including diabetic nephropathy, lupus nephritis, IgA nephropathy and membranous nephropathy. Podocyte injury in these patients is often multifactorial and can arise due to a variety of factors, including inflammatory and metabolic factors. Another type of glomerular injury that can give rise to the development of secondary FSGS is glomerular hyperfiltration. Glomerular hyperfiltration is observed in patients with hypertensive nephropathy and patients with a discrepancy between renal mass and total body volume either because of reduced renal mass (due to e.g. a nephrectomy or a congenital monokidney) or increased body mass (obesity). Although these risk factors are associated with hyperfiltration and secondary FSGS, one should be cautious with a pronto diagnosis of secondary FSGS. After all, with an obesity prevalence of approximately 25%,[83] a patient with obesity could very well present with primary FSGS.

Two other important causes of secondary FSGS are drug toxicity and viral infections. Several medications have been associated with the development of FSGS (Table 3).[74,84,85] The mechanism of (podocyte) injury due to most of these substances remains to be determined. Among the infectious causes, human immunodeficiency virus (HIV) has the strongest association with FSGS. Classical presentation of HIV-associated FSGS is overt proteinuria, rapidly progressing renal insufficiency and collapsing FSGS in the renal biopsy. The pathophysiological mechanism of HIV-associated FSGS likely involves direct infection of the podocyte via binding of the HIV virus to CD209 receptors. In addition, the HIV protein Tat can access podocytes via heparan sulfate proteoglycans and cholesterol-enriched lipid rafts.[86] Cell cycle dysregulation and aberrant podocyte cell cycle re-entry appear to be a hallmark of the pathogenesis of HIV-associated collapsing nephropathy, although the exact mechanisms remain incompletely understood.[87] Besides HIV, there are several other viral infections that are associated with FSGS. These are listed in Table 3.[74]

Pathogenesis of FSGS – the injured podocyte

The hallmark of all variants and forms of FSGS is podocyte injury. A substantial part of our knowledge on podocyte injury in FSGS originates from animal models. Studies on puromycin nephropathy models for FSGS showed us that one of the earliest morphological changes in the development of FSGS is podocyte foot process effacement, reflecting podocyte cytoskeleton changes in response to podocyte injury (Figure 4A).[88,89] In these animal models, podocyte foot process effacement occurs concomitantly with proteinuria, but appears before the development of matrix accumulation in the glomeruli. Biopsy findings in patients with FSGS are in line with these animal studies: foot process effacement occurs in glomeruli affected by FSGS lesions as well as in glomeruli (yet) unaffected by FSGS lesions.[47] In addition, the occurrence of foot process effacement before the development of FSGS lesions in recurrent FSGS,[77] suggests that podocyte foot process effacement is an early event in the development of human FSGS. Several animal studies have shown that progressive podocyte depletion is an important subsequent step in FSGS development.[90] Podocyte depletion can arise due to various mechanisms, including cell death due to apoptosis, mitotic catastrophe and dysfunctional cellular adhesion to the GBM. Which of these mechanisms is the major contributor to podocyte loss in FSGS remains to be determined. Again, podocyte depletion as observed in animal models is also a feature in human FSGS.[91] Being terminally differentiated cells, podocytes do not have the capacity to divide and replenish the pool of lost podocytes. There are several studies providing evidence that parietal epithelial cells covering the capsule of Bowman serve as a reservoir for depleted podocytes. Kriz *et al.* showed that cellular bridges are formed between the denuded basement membrane and Bowman's capsule in the early development of FSGS.[92] These cellular

Table 2. Genetic mutations and susceptibility genes associated with FSGS

Mendelian and mitochondrial inheritance		Susceptibility genes
Slit pore or cytoskeleton molecules	Other	
NPHS (nephrin)	*COL4A3*	*APOL1*
NPHS2 (podocin)	*COL4A4*	*PDSS2*
CD2AP	*COL4A5*	*WNK4**
PTPRO (GLEPP1)	*XP05*	*KANK1**
MYO1E	*NXF5*	*IL36G**
ACTN4	*PAX2*	*ARHGEF17**
INF2	*PLCE1*	
AHRGP24	*TTC21B*	
AHRGDIA		
TRPC6		

*Validation in a larger patient population required

Table 3. Medication and viral infections associated with the development of FSGS

Medication	Viral infections
Lithium	Human immunodeficiency virus
Interferon-α, -β, or -g therapy	Cytomegalovirus
Anti-vascular endothelial growth factor therapy	Parvovirus B19
Bisphosphonates	Epstein-Barr virus
Sirolimus	

bridges, also called synechiae, were originally thought to be podocytes. Subsequent studies showed that parietal epithelial cells are also important components of these synechiae (Figure 4B).[93,94] Smeets *et al.* demonstrated in three different models of FSGS that, following primary injury, activated parietal epithelial cells migrate to the glomerular tuft via cellular adhesions. Via this entry site, the activated parietal epithelial cells invade the affected glomerulus, deposit extracellular matrix and trigger mesangial cells to produce extracellular matrix (Figure 4C).[93] Moreover, cells positive for CD44, a marker of activated parietal epithelial cells, contribute to the development of hyperplastic lesions in a model for collapsing FSGS.[95] Thus, although parietal epithelial cell migration to the glomerular tuft might be aimed at replenishing lost podocytes, it turns out to be a failing compensatory mechanism and a major contribution to the development of sclerotic lesions in the pathogenesis of FSGS (Figure 4D). Signs of these early events in the development of FSGS can be observed in patients as well. Glomerular CD44-positive cells can be identified in the earliest lesions of FSGS while in patients with MCD, no CD44-positive cells are observed.[96,97] This shows that parietal epithelial cell activation is an early event in the development of FSGS. Moreover, these cells also appear to contribute to matrix production in FSGS: studies have shown that sclerotic lesions in FSGS are composed of extracellular matrix molecules derived from mesangial cells and (parietal) epithelial cells.[98,99] Still, the underlying mechanisms leading to matrix production by these cells remain incompletely understood.

The previous paragraph shows that many of the individual steps of FSGS development, from podocyte injury to the development of glomerulosclerosis, are known. However, the transitions between the individual steps remain poorly understood. An important missing link is why podocyte injury in FSGS is progressive. Animal models have shown that the initial changes observed in podocytes are reversible, indicating that podocyte injury alone is not sufficient for the development of FSGS. This is also reflected by the clinical entity MCD: podocyte changes in MCD are similar to those in FSGS, but patients with MCD generally do not show progressive podocyte loss and the development of glomerulosclerosis. The fact that podocyte injury, as observed in MCD and early stages of FSGS, can be reversible, has led to the notion that podocyte injury in FSGS must progress to a point of no return. However, the factors contributing to progressive podocyte injury in FSGS are incompletely understood. In addition, it remains unclear how much podocyte damage is required for glomerulosclerosis to develop. A study by Wiggins *et al.* illustrated that the extent of podocyte depletion determined whether FSGS would develop in a diphtheria toxin model.[90] This suggests that podocyte depletion is a major contributor to the development of FSGS. However, it does not sufficiently reflect the situation in humans, since FSGS lesions can also develop in cases with limited podocyte loss. In

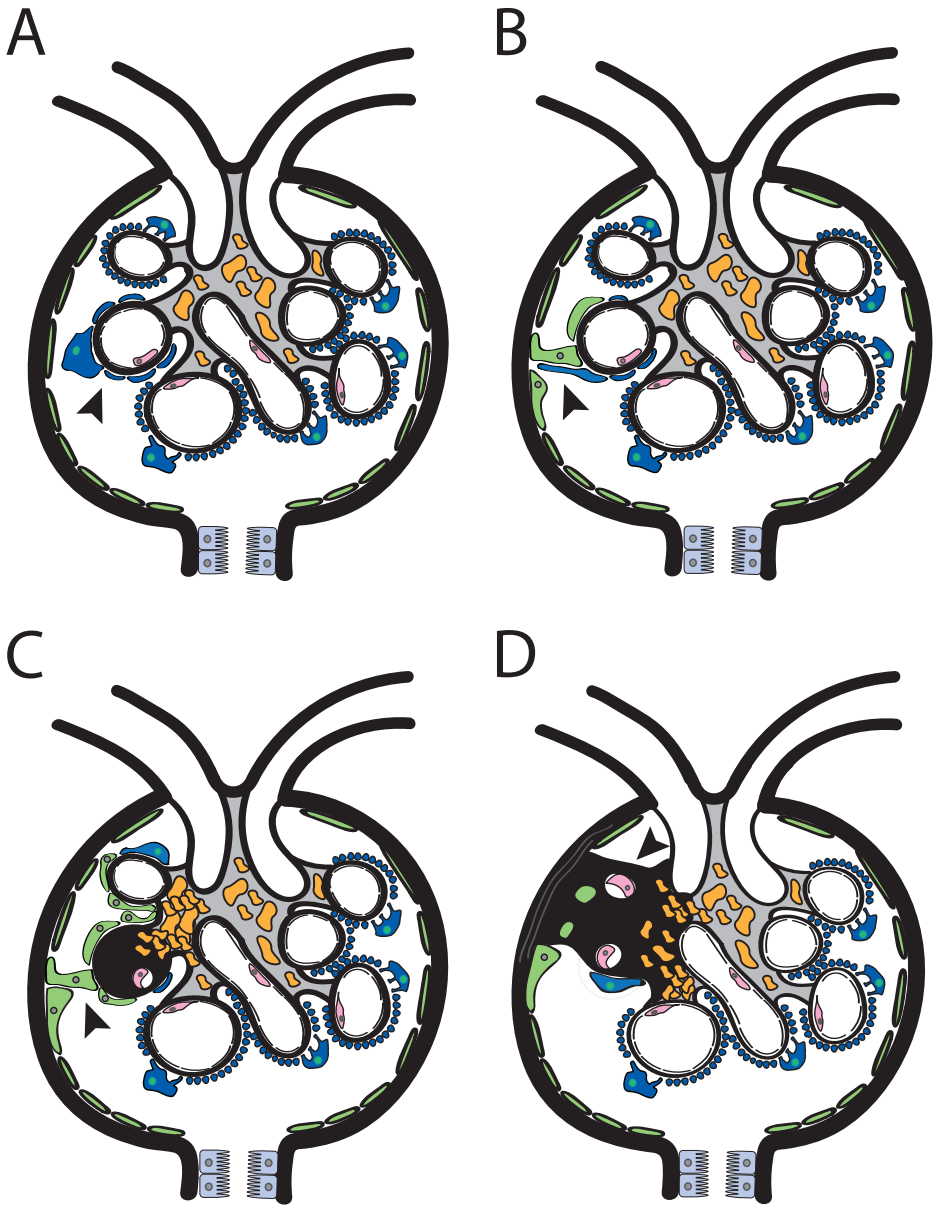

Figure 4. Stepwise schematic representation of FSGS pathophysiology.

(A) Initial podocyte injury leading to morphological changes of podocytes with foot process effacement (arrowhead). (B) Podocyte injury continues and may lead to podocyte detachment and denudation of the glomerular basement membrane. Parietal epithelial cells become activated and form cellular bridges to the affected portion of the glomerular tuft in order to cover the bare basement membrane (arrowhead). (C) Infiltrating parietal epithelial cells (arrowhead) fail to successfully replace the podocytes and start to produce extracellular matrix. Mesangial cells are stimulated to proliferate and start to produce matrix as well. (D) Excessive matrix production leads to the occlusion of capillary lumina. Arrowhead indicates an FSGS NOS lesion.

conclusion, more research into the pathogenesis of FSGS is necessary for a more thorough understanding of its pathophysiological mechanisms.

The development of recurrent FSGS

Recurrent FSGS occurs in cases of primary FSGS in which a systemic or circulating factor is likely involved. It develops in approximately 30% of renal transplant recipients with a native diagnosis of primary FSGS.[75,76] Recurrent FSGS is a remarkable phenomenon in which the native disease can recur in the transplanted kidney in a matter of hours. One striking case illustrates recurrent FSGS perfectly: a 27-year-old male with ESRD due to primary FSGS received a kidney transplant from his younger sister. Prior to transplantation the patient underwent plasmapheresis in order to minimize the risk of recurrence. However, heavy proteinuria developed on the second post-transplant day. A kidney biopsy a few days later showed findings consistent with MCD with extensive foot process effacement on electron microscopy, which was considered early recurrence of the native disease. Because of persistent proteinuria and worsening kidney function, the allograft was removed two weeks after transplantation. The treating physicians made the exceptional decision to look for a willing recipient of the failing transplant. A 66-year-old male with ESRD due to diabetic nephropathy agreed to the procedure. The retransplanted kidney regained function in the absence of proteinuria immediately after transplantation and 8 months later renal function was still excellent. Moreover, histological changes that appeared in the first recipient were completely reversed.[100] This case provides valuable insights into the pathogenesis of recurrent, and also native FSGS. It supports the role of a systemic factor that, when eliminated, leads to full recovery of kidney function. Intriguingly, plasmapheresis did not lead to a sufficient elimination of this systemic factor. Secondly, it shows that injury to the podocyte that led to the development of FSGS in the native kidney, is indeed reversible when the stressor is removed in an early phase of the disease.

Not all cases of recurrent FSGS develop this rapidly: it can take up to two years after transplantation for recurrent FSGS to develop.[77] Later cases of proteinuria are usually considered due to chronic allograft dysfunction, although it is difficult to assess this with certainty. Kidney biopsies performed soon after recurrence, within four to six weeks, mostly show the characteristics of MCD, with overt foot process effacement and without significant light microscopic changes. Biopsies taken later in the course of the disease may show evidence of FSGS with a high fidelity to the original variant of FSGS.[101,102]

Treatment of FSGS

FSGS still presents a major therapeutic challenge. The current therapy for primary FSGS is based on immunosuppressive agents. The use of immunosuppressive

therapy as part of the early treatment regimen leads to improved renal outcome.[103] The first line-immunosuppressive treatment is based on glucocorticosteroids. Response to glucocorticosteroids differs depending on age, race and variant of FSGS, but an overall initial complete remission rate is estimated at 20-25%.[65] Increasing doses of corticosteroids can help reaching complete or partial remission. Steroid resistance is associated with poor renal outcome in both adults and children and many patients that do respond to steroids remain steroid dependent.[65,104,105] Second line immunosuppressive agents include cyclophosphamide, cyclosporine, mycophenolate, tacrolimus and rituximab. Cyclosporine is considered to be among the most successful immunosuppressive agents in the treatment of FSGS.[106] Nevertheless, its efficacy is greatly dependent on previous prednisone response, with a success rate of approximately 70% in steroid-responsive cases and 30% in steroid-resistant cases. Niaudet *et al.* and Tahar *et al.* showed that complete remission rates in children are significantly higher when steroids are given in combination with cyclosporine.[107,108] Tacrolimus has also proven to be effective in treating steroid resistant FSGS and appears to result in less renal toxicity compared to cyclosporine. Although cyclophosphamide is widely used in the management of FSGS, the reported therapeutic effects are limited. The same holds true for mycophenolate.[65] One of the most recent advances in the treatment of FSGS is the monoclonal antibody rituximab. Several studies support the use of rituximab in frequently relapsing or steroid dependent MCD. Although some retrospective studies describe beneficial effects of rituximab in FSGS, the currently available evidence is insufficient to support additional value for rituximab in the management of FSGS.[109-113] Finally, plasma exchange therapy has emerged as a promising therapeutic option and is especially applied for the prevention and the management of recurrent FSGS.[114,115]

Part 3: new players in FSGS

Our understanding of FSGS has increased significantly over the last couple of decades. However, there are still many unknowns surrounding this heterogeneous pathological entity. One important field of research has focused on the missing links in podocyte pathobiology in FSGS: which factors elicit and/or contribute to the progression of podocyte injury and the development of sclerotic lesions? Although our knowledge of the podocyte has increased massively since its discovery, much about this unique cell type with its exceptional morphology is still unknown. Advances in genetic research have already led to important discoveries and recent developments will open up more possibilities for the identification of new target genes. Nevertheless, the answers to these questions might not only lie in podocyte biology. The discovery of the parietal epithelial cell as a major contributor to FSGS development has paved the way for other cell types to be explored. In addition,

research on disease mechanisms beyond glomerular biology, such as the immune system, that were previously unrecognized, has led to promising new insights in the pathogenesis of FSGS. In the following section, several encouraging developments in the field of FSGS research will be explored. First, the opportunities of genetic analysis of an FSGS animal model will be discussed. The second part will focus on the current knowledge of the role of the complement system in FSGS. Lastly, the evidence for a role of the glomerular endothelial cell in FSGS will be explored.

Lessons from the Munich Wistar Frömter animal model

Animal models have proven to be of major help in identifying new pathological pathways. Especially for the investigation of the course of events, animal models are indispensable. Many problems we are faced with in our attempts to identify new players in a disease process in humans are overcome by animal models: they demonstrate high genetic and environmental homogeneity and can easily be followed over time. One unique model for studying the spontaneous development of proteinuria, podocyte injury and FSGS is the Munich Wistar Frömter (MWF) rat model. MWF rats have an inherited deficit in nephron number and a high number of superficial glomeruli.[116,117] They spontaneously develop albuminuria after 6-8 weeks of age, which is followed by the development of hypertension, overt proteinuria and glomerular sclerotic lesions with high resemblance to human FSGS.[116,118] Overt proteinuria in these rats coincides with redistribution of slit pore markers, foot process effacement and progressive podocyte loss, indicating that podocyte pathology is an important aspect of the renal phenotype.[118-120] The MWF rat has proven to be a valuable model for identifying and studying new factors in the development of FSGS. IJpelaar *et al.* showed that the development of proteinuria is accompanied by focal and segmental loss of podoplanin protein expression and *de novo* protein expression of the dedifferentiation marker desmin.[118] Investigating the specific genetic background of these rats might reveal previously unknown pathways involved in disease development. Linkage analysis has identified a locus on chromosome 6 that is responsible for the renal phenotype.[121] A more in depth analysis of this locus will provide us with answers to the question what genetic alterations make these rats vulnerable to podocyte injury and the development of FSGS.

The complement system and its role in FSGS

The complement system describes an operation system of over 20 proteins that can be sequentially activated in an enzymatic cascade (Figure 5). At the beginning of the last century, the complement system was discovered as the effector arm of the innate immune system and a major player in the first line host defense mechanisms.[122,123] However, the significance of the complement system reaches far beyond its role

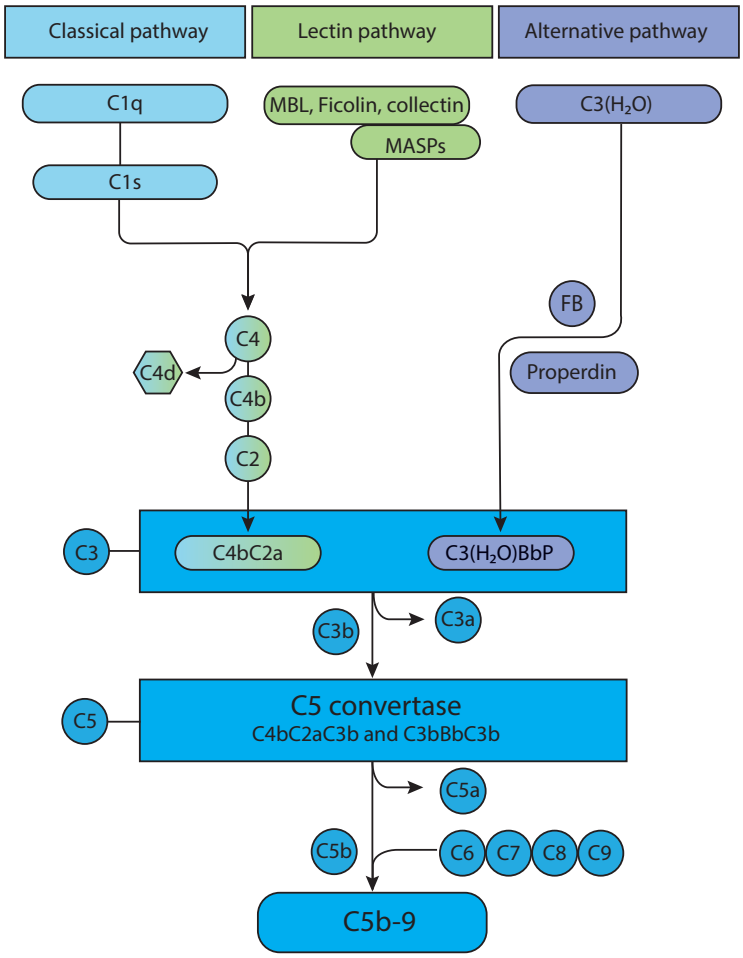

Figure 5. Schematic representation of the complement system

The complement system can become activated via three distinct pathways, all of which lead to the activation of a final common pathway. The classical route of the complement system is typically activated by C1q binding to antibodies. This induces a conformational change leading to activation of C1s. C1s then cleaves C4 into C4a and C4b. During this process, an inactive component (C4d) is formed and covalently binds to the cell surface or matrix. C4b also attaches to the activator surface and then binds C2. C2 cleavage into C2b and C2a facilitates the formation of the complex C4b2a, a C3 convertase that serves as activator of the common pathway of the complement system. The lectin route of the complement system is activated by mannose groups on pathogen cell surfaces that are recognized by mannose-binding lectin, ficolins or collectins. Together with mannose-binding lectin–associated proteases 1 or 2, this complex leads to the cleavage of C4 and subsequently to the formation of C4b2a, similar to the classical pathway. The alternative route is initiated by the covalent binding of a small amount of C3b to hydroxyl groups on cell-surfaces and is activated by low-grade cleavage of C3 in plasma. C3b binds factor B to form C3bB, the C3 convertase of the alternative pathway. Under physiological conditions, this C3 convertase has a very short half-life. The binding of properdin can stabilize the structure. The C3 convertase enzymes cleave many molecules of C3 to C3b, which bind covalently around the site of complement activation, leading to an amplification of the cleavage rate and C3 convertase formation. When additional C3b binds to C3 convertase formed in any of the three pathways, a C5 convertase enzyme is formed. C5 convertase cleaves C5 into the anaphylatoxin C5a and C5b. The subsequent assembly of C5b, C6, C7, C8 and several molecules of C9 gives rise to the pore-forming complex C5b-9, or membrane attack complex, the final effector molecule of the complement system.[123,124]

in host defense. Over the years, many other functions have been ascribed to the complement system including regulation of growth, clearing of apoptotic cells and apoptosis derived self-antigens, clearance of immune complexes, recognition of neoepitopes presented by injured self-cells and orchestrating the subsequent autoinflammatory response.[123,124] The complement cascade can be activated via three distinct pathways: the classical pathway, the lectin pathway and the alternative pathway. The activation of any of the three pathways leads to the formation of C3 convertases. The C3 convertases, in turn, cleave C3 which triggers the activation of the final common pathway via the formation of C5 convertases. The cleavage of C5 forms the molecule C5b. By recruiting the complement components C6 through C9, C5b forms the membrane attack complex (MAC or C5b-9), the final effector complex of the complement cascade.

Although the complement system is essential for defense and repair, disturbances in the activation or regulation of the cascade can lead to disease. The kidney, and in particular the glomerulus, is frequently affected in complement-mediated disease. The glomerulus is a preferred site for the deposition of immune complexes due to the presence of high pressure and possibly the fenestrated endothelium.[125] The field of nephrology has been long aware of the role of complement in immune complex-mediated diseases. However, the attention is shifting and the role of complement in non-immune complex-mediated glomerular diseases has become a new topic of research.[126]

The long prevailing view has been that FSGS is not a complement mediated disease. The classical tool for assessing complement involvement in glomerular disease is the immunofluorescent staining of IgG, IgA, IgM, C3 and C1q on frozen sections of a renal biopsy. In routine diagnostics, findings in renal biopsies with FSGS do not show a classical staining pattern as known from other complement-mediated renal diseases. However, biopsies with FSGS do not necessarily have to be negative: C3 and IgM are often positive in FSGS lesions and positivity of C1q can occur as well.[49] However, the staining in sclerotic areas is generally not considered a positive finding in routine diagnostics, but rather viewed as nonspecific trapping of complement macromolecules in sclerotic areas. This view of nonspecific trapping of complement components in sclerotic areas with no apparent diagnostic value has led to the assumption that complement activation does not play a role in FSGS. Although research on complement activation in FSGS is scarce, several observations suggest the contrary. Firstly, Gephardt *et al.* described depositions of IgM and/or C3 in non-sclerotic glomeruli in 40% of patients.[127] By definition, these depositions cannot be regarded as the result of nonspecific entrapment. Secondly, a study investigating plasma and urine samples of 19 patients with primary FSGS showed

elevated levels of complement activation products in both plasma and urine.[128] Thirdly, Turnberg *et al.* have shown that deficiency of complement components leads to a reduction in glomerulosclerosis in an FSGS animal model, while Morigi *et al.* showed that deficiency of the complement cascade inhibitor factor H accelerates the development of glomerulosclerosis.[129,130] Moreover, a study investigating IgM depletion in the setting of FSGS showed that IgM induced complement activation is required for the development of sclerosis in Adriamycin nephropathy.[131] Although the aforementioned results suggest a role for complement activation in the pathogenesis of FSGS, studies on complement activation in the kidneys of patients with FSGS and the relation to glomerulosclerosis are still missing.

No cell is an island: the glomerular endothelium in FSGS

FSGS has long been considered a disease of the podocyte. Yet, due to our increasing knowledge of the GFB and the relationship between its different components, the idea that glomerular diseases are the result of dysfunction of a single cell type has taken leave. For several glomerular diseases, the attention has already shifted from one cell type of the GFB to the other. For instance, whereas preeclampsia was originally thought of as a disease of primarily the glomerular endothelium, the focus has now moved to the podocyte.[132] Also in diabetic nephropathy, the involvement of both glomerular endothelial cells and podocytes in disease development are acknowledged.

The glomerular endothelium can be affected in glomerular disease by direct injury or by disturbance of the glomerular microenvironment. As has been illustrated in the prologue, glomerular endothelial cells and podocytes, the two cellular components of the GFB, communicate with each other from the moment of their earliest development. Proper endothelial and podocyte differentiation depends on this close contact and impaired signaling during glomerular development can lead to an immature, non-functional glomerulus.[133] Crosstalk between podocytes and endothelial cells is not only of importance during glomerulogenesis, but remains crucial long after the glomerulus has formed. Studies have indicated that impaired crosstalk between these two cell types can give rise to several glomerular diseases, including preeclampsia, diabetic nephropathy and thrombotic microangiopathy induced by anti-vascular endothelial growth factor A (VEGFA) therapy. Various signaling pathways are implicated to play a role in podocyte-endothelial communication, as has been comprehensively reviewed by Dimke *et al.*[134] However, the role of most of these pathways in the development of glomerular disease is still incompletely understood.

A role for the glomerular endothelium might be implicated in FSGS as well. In 1998, Salwa *et al.* reported endothelial vacuolization in early stages of FSGS.[135] One of the few studies directly focusing on endothelial injury in primary forms of FSGS, compared ultrastructural endothelial changes between patients with FSGS and patients with MCD and found that subendothelial widening was markedly increased in patients with FSGS.[136] In addition, it was reported that endothelial morphological changes were associated with the development of secondary FSGS in patients with idiopathic membranous nephropathy.[137]

Besides morphological changes in the glomerular endothelium, other aspects of endothelial injury have also been described in relation to FSGS. Zhang *et al.* showed that serum markers of endothelial dysfunction, such as soluble thrombomodulin and von Willebrand factor, were higher in patients with primary FSGS compared to controls.[138] These markers decreased as patients went into remission. Yet, it remained uncertain whether the increase of endothelial biomarkers was due to changes in the glomerulus or reflected a systemic response due to the nephrotic syndrome. A recent study by Menon *et al.* provided evidence for glomerular endothelial cell activation in patients with FSGS using single cell transcriptomic analysis.[139] Interestingly, of the 10 different glomerular diseases that they investigated, FSGS showed the highest glomerular endothelial cell "activation scores". Moreover, higher levels of certain transcripts were associated with lower proteinuria remission rates, linking endothelial dysfunction in FSGS to clinical outcome.

Two specific variants of FSGS display a stronger association with endothelial injury than others. The cellular variant of FSGS is per definition characterized by intracapillary changes. In addition, an association between endothelial injury and the collapsing variant of FSGS has come to light. Collapsing FSGS is a relatively common secondary phenomenon in biopsies of cases with diabetic nephropathy.[140] Especially the cases with severe vascular injury are prone to develop secondary collapsing FSGS. Collapsing FSGS is also common in renal thrombotic microangiopathy.[141] These two studies also showed that collapsing FSGS was not only associated with glomerular endothelial injury, but also with injury to the renal arterioles. Collapsing FSGS is linked to endothelial injury due to its association with renal diseases characterized by microvascular injury, but also because it can be induced by anti-VEGFA therapy in both animals and patients.[5,142] As anti-VEGFA therapy is a known cause of endothelial injury as well as podocyte injury, collapsing FSGS in these cases might be the result of endothelial injury, podocyte injury, or a combination of both. The findings from the human FSGS studies are supported by several experimental studies. Apoptosis of glomerular endothelial cells was increased during the development and progression of glomerulosclerosis in a remnant-kidney

model.[143] In a later study, endothelial cell injury after administration of Adriamycin nephropathy was reported. Interestingly, endothelial changes already occurred 24 hours after Adriamycin administration, while podocyte morphological changes only occurred after 7 days.[144] The presence of endothelial morphological changes prior to podocyte changes was recently confirmed in the constitutively active transforming growth factor β (TGF-β) receptor model of glomerulosclerosis.[15] Lastly, Kreutz. *et al.* reported data on glomerular endothelial cell replenishment from progenitor cells after glomerular injury in the MWF rat model, pointing towards an endothelial rearrangement/repair mechanism taking place in the glomeruli of proteinuric MWF rats.[145]

Although these results shed a different light on the concept of FSGS as solely a disease of the podocyte, the role of endothelial or microvascular injury in FSGS and especially primary FSGS is not sufficiently clear yet. The presence of both endothelial and podocyte changes in FSGS suggests that altered crosstalk between dysfunctional endothelial cells and podocytes might also be a crucial factor in the development of FSGS, in addition to isolated injury to either one of these cell types. Experimental evidence for altered crosstalk between podocytes and endothelial cells in FSGS comes from a study investigating a mouse model of podocyte injury and glomerulosclerosis induced by constitutive activation of the TGF-β receptor in podocytes.[16] In this study, the authors focused on the endothelin-1 (ET-1) system as mechanism of podocyte-endothelial interaction. ET-1 has been implicated in glomerular podocyte-endothelial crosstalk before. Originally, ET-1 was described as an endothelial-derived molecule with very potent vasoconstricting properties, acting upon the smooth muscle cells of the vessel wall. However, it is now evident that the small peptide exerts many effects throughout the body and is produced by numerous cell types.[146] In the glomerulus, ET-1 is produced by both podocytes and endothelial cells. In the experimental study by Daehn *et al.*, the authors showed that podocyte-derived ET-1 caused mitochondrial oxidative stress in glomerular endothelial cells via stimulation of the endothelin receptor A (ET_AR) expressed on endothelial cells. Specific inhibition of ET_AR significantly reduced glomerulosclerosis and serum creatinine and increased the number of podocytes. Furthermore, in a coculture model of endothelial cells and podocytes, Daehn *et al.* specifically showed that stimulation of endothelial cells with ET-1 led to an subsequent increase in podocyte apoptosis. Blocking ET-1 signaling in endothelial cells abolished these effects.[16] These results indicate that overt TGF-β signaling in podocytes results in podocyte loss and glomerulosclerosis only via crosstalk with glomerular endothelial cells, presumably via the endothelin system. In a follow-up study by the same research group, the authors further investigated the effects of ET-1 on endothelial cells. Prior to podocyte foot process effacement, they observed degradation of

the endothelial surface layer, a crucial component of the glomerular filtration barrier, which coincided with the development of albuminuria.[15] Hence, they concluded that activation of ET-1-ET_AR interaction on endothelial cells contributes to the pathogenesis of primary podocytopathies in experimental segmental glomerulosclerosis. These experimental studies indicate that the endothelial cell might already be involved in the early pathogenesis of FSGS, and suggest that podocyte-endothelial crosstalk in FSGS is a promising mechanism to explore further.

Part 4: this thesis

Our understanding of FSGS has greatly improved since its discovery. Advances have been made concerning the causes of initial podocyte injury, the different stages of podocyte damage and the final development of the sclerotic lesions. However, several crucial questions remain unanswered. An important aspect of FSGS research has focused on a circulating factor that initiates the development of FSGS. When found, this discovery will herald a major breakthrough in the field of FSGS research. Another major aspect of FSGS biology that still puzzles investigators and clinicians is how, why and when podocyte injury in FSGS becomes progressive, finally leading to irreversible loss of podocytes and chronic FSGS lesions. Many of the stages of podocyte injury are known to us, but for most of these stages the triggers remain undiscovered. The question remains whether a single factor is the cause of the disease entity primary FSGS. Most clinical observations suggest that it likely arises due to a variety of factors. The hiatus in our understanding of how, why and when podocyte injury in FSGS becomes progressive has also held back advancements in treatment. Identifying new biological pathways could offer great opportunities for treatment development. Especially transferring pathways identified in animal models to the clinical setting could be of major value. Ample studies have demonstrated that results from animal studies often do not correspond with the human situation. Evidence of the involvement of these mechanisms in humans, albeit indirect, could be of great assistance in guiding new treatment possibilities and future research. In addition to new treatment options, being able to identify progressive podocyte injury and FSGS development in an early stage would aid in optimizing therapeutic results.

This thesis describes new players in the development of FSGS with a special emphasis on those aspects involved in the progression of podocyte injury. In the first part of this thesis, the podocyte has a central position. **Chapter 2** concentrates on clinical markers to determine progressive podocyte injury in the setting of patients with MCD. Here, we investigate whether glomerular nephrin loss in renal biopsies could

serve as a marker for disease progression in this patient population. In **Chapter 3** and **Chapter 4** we explore the genetic background of the MWF rat model in order to determine how its genetic predisposition contributes to the development of podocyte injury, proteinuria and FSGS. In **Chapter 3**, an extensive exploration of the genetic background of the MWF rat strain is presented. Furthermore, the involvement of one of the target genes, that codes for transmembrane protein 63c, in the development of proteinuria and FSGS, is analyzed in more detail. **Chapter 4** focuses on the role of prostaglandin reductase 2, another target gene identified in the MWF rat model, in podocyte injury.

In the second part of this thesis we explore new players in FSGS that stretch beyond podocyte biology. **Chapter 5** focusses on complement activation in glomeruli of patients with FSGS. In this study, we investigated whether glomerular complement deposition precedes the development of FSGS lesions and whether this represents local complement activation. In **Chapter 6,** we set out to validate the mechanism of altered podocyte-endothelial crosstalk via ET-1 signaling and oxidative stress in human FSGS, as was proposed in experimental studies. Finally in **Chapter 7**, the landscape of microvascular lesions that can be observed in patients with FSGS is described, with a special focus on collapsing FSGS.

A better understanding of how these investigated factors interact with injured podocytes will result in new insights regarding the progression of podocyte injury and glomerulosclerosis in FSGS, paving the way for future research into different pathogenic pathways and new therapeutic opportunities.

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