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REVIEW

Molecular genetic analysis of podocyte genes in focal segmental glomerulosclerosis—a review

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Abstract This review deals with podocyte proteins that play a significant role in the structure and function of the glomerular filter. Genetic linkage studies has identified several genes involved in the development of nephrotic syndrome and contributed to the understanding of the pathophysiology of glomerular proteinuria and/or focal segmental glomerulosclerosis. Here, we describe already well-characterized genetic diseases due to mutations in nephrin, podocin, CD2AP, alpha-actinin-4, WT1, and laminin β2 chain, as well as more recently identified genetic abnormalities in TRPC6, phospholipase C epsilon, and the proteins encoded by the mitochondrial genome. In addition, the role of the proteins which have shown to be important for the structure and functions by gene knockout studies in mice, are also discussed. Furthermore, some rare syndromes with glomerular involvement, in which molecular defects have been recently identified, are briefly described. In summary, this review updates the current knowledge of genetic causes of congenital and childhood nephrotic syndrome and provides new insights into mechanisms of glomerular dysfunction.

Keywords Nephrotic syndrome · Focal segmental glomerulosclerosis · Podocytes · Nephrin · Podocin · TRPC6 · PLCE1

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The glomerular filtration barrier

The glomerulus consists of a cluster of capillaries appearing in a looped formation supported by mesangial cells. While blood plasma passes the glomerular capillary loops, the local intracapillary pressure drives plasma through the glomerular filtration barrier which consists of three layers (Fig. 1).

First, the glomerular endothelial cells separate the blood and tissue compartments. The endothelial cells are highly flattened cells and regulate vasomotor tone and hemostasis [4]. The role of the endothelial cells in selective filtration seems not to be substantial since they are highly fenestrated and highly permeable to water and small solutes. However, a recent study has shown that morphological alterations in the endothelial cell glycocalyx, making the cell surface extremely negatively charged, have functional consequences for glomerular permeability [54]. Using the GAG-degrading enzyme chondroitinase decreasing the thickness of the endothelial cell glycocalyx resulted in an increase of albumin clearance [54].

The endothelium is completely surrounded by the second layer, the glomerular basement membrane (GBM). This dense structure of extracellular matrix components provides structural support for the capillary wall necessary

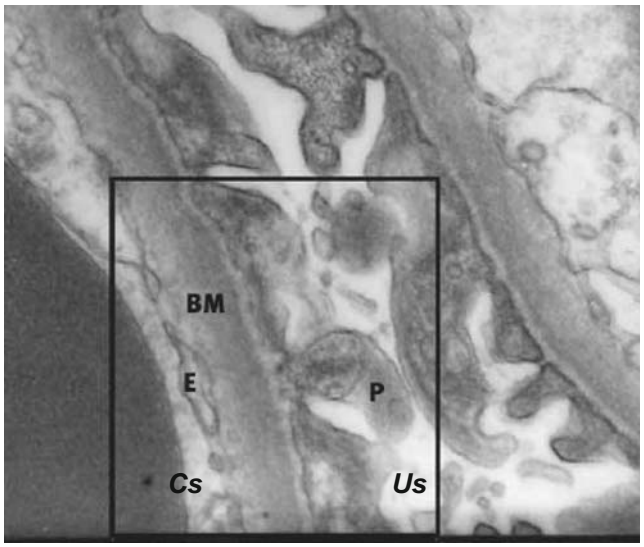

Fig. 1 A cross-section (electron microscopy, original magnification ×30,000) of the glomerular filtration barrier with the capillary space (*Cs*), urinary space (*Us*), endothelial cells (*E*), glomerular basement membrane (*BM*), and podocytes (*P*)

to maintain local high blood pressure. The main components of the GBM (collagen type IV, laminins, nidogen, and proteoglycans) contribute to the selective permeability of the GBM based on size and charge [72]. In the past years, many studies were focussing on the structure of the GBM because it was considered to be the leading part of the glomerular filtration barrier. Although structural abnormalities in the GBM may lead to proteinuria and hematuria, as it occurs in Alport's syndrome (mainly due to collagen IV α5 chain mutations, but also collagen IV α3 and α4 chains) or Pierson's syndrome (due to laminin β2 mutations) [8, 146], the discovery of several novel proteins important for glomerular permeability made the podocytes the favorite candidate for constituting the main part of the glomerular filtration barrier.

The podocytes, or visceral epithelial cells, represent the third layer. They are highly specialized, terminally differentiated cells with cytoplasmic extensions, the so-called foot processes. Podocytes have an important role in size and charge selective permeability, but also in synthesizing and maintaining the GBM [98]. Furthermore, the fenestration of endothelial cells depends most notably on vascular endothelial growth factor A secreted from differentiated podocytes [4].

The foot processes of the podocytes attach to the outer surface of the GBM through cell membrane receptors (α3β1 integrins linked to talin, vinculin, and paxillin, and α- and β-dystroglycans linked to utrophin; see Fig. 2) [68]. Adjacent foot processes interdigitate, forming a pore of about 25–40 nm in width. This pore, or slit, is covered by a membrane with a "zipper-like" structure [108]. Recently, the structure of the slit membrane became the focus of many studies, and although the complete structure is not elucidated yet, several new proteins were found to be important for its function. Many components of the slit membrane are involved in the pathogenesis of (nephrotic range) proteinuria.

Nephrotic syndrome—focal segmental glomerulosclerosis

A nephrotic syndrome (NS) is defined by the occurrence of heavy proteinuria, edema, and hypoalbuminemia, and is classified as steroid-sensitive or steroid-resistant. In children NS occurs most commonly at a young age, with a peak incidence at 2 years. In most cases (~80%, according to the International Study of Kidney Disease in Children (1967–1974), ISKDC) patients have minimal change NS (MCNS), which is characterized by responsiveness to steroid treatment accompanied with more or less frequent relapses. In 5–10% of NS patients (ISKDC) focal segmental glomerulosclerosis (FSGS) is found [83, 128]. Less frequent histologic lesions are mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, and membranous glomerulopathy [83].

FSGS is a histological finding commonly seen in a large variety of conditions with different underlying causes.

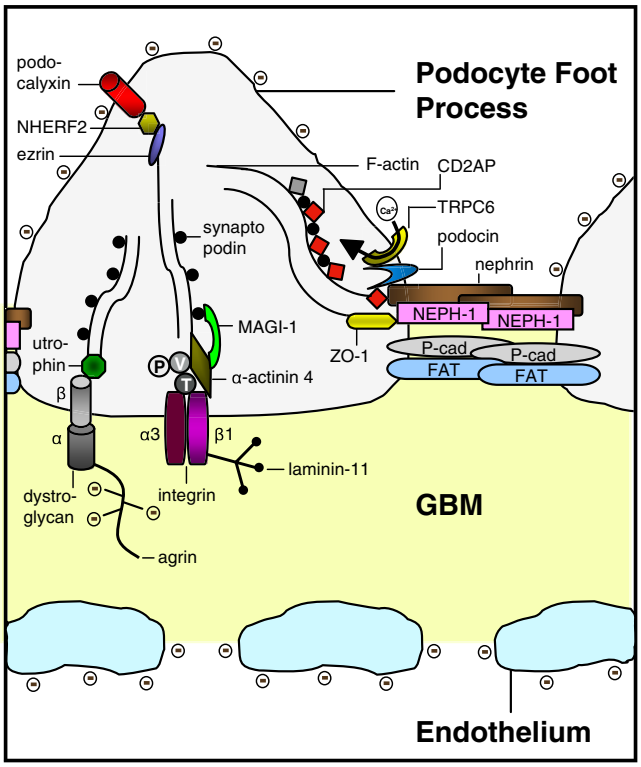

Fig. 2 Molecular anatomy of the podocyte slit membrane, schematic representation. *P* paxillin, *V* vinculin, *T* talin, ⊖ negatively charged glycogalyx. Modified from [86], with permission

These conditions share the focal (only some of the glomeruli are involved) segmental (only part of an entire glomerulus is involved) sclerosis of the glomerular capillary tuft and manifests with proteinuria. As the disease progresses, the sclerosis has a more diffuse and global pattern. The biopsy may show mesangial deposits of immunoglobulin M or complement C3 and hyaline deposits all over the capillary loops [143]. Podocyte alterations (like foot process effacement) are most notably in FSGS.

Recently, FSGS has been classified in five subcategories: collapsing variant, tip lesion variant, cellular variant, perihilar variant, and FSGS not otherwise specified (NOS) [20, 22 for further reading]. Whether subdividing FSGS in different subcategories gives insight in clinical features and renal outcome is a subject of several reports recently reviewed by D'Agati [21]. Collected data from adult patients with biopsy proven FSGS showed a predisposition of younger and more often African–American patients for the collapsing variant of FSGS [131]. The collapsing and tip variants usually manifest with more severe proteinuria compared to the perihilar and NOS variants. Patients with the last two morphologic variants tend to have higher blood pressure and pathologically more arteriosclerosis [131]. As far as renal outcome concerns, the patients with tip lesion variant of FSGS seem more often to achieve complete remission, whereas the collapsing variant of FSGS has worse renal survival rates [131]. It is not excluded that different morphologic variants represent different stages of disease progression in FSGS.

FSGS commonly progresses to end-stage renal disease (ESRD) requiring dialysis or renal transplantation. Post-transplant recurrence occurs in 35–40% of patients with idiopathic FSGS [2, 23], mainly a few days after transplantation, and is often sensitive to plasmapheresis and cyclophosphamide [3, 58, 116] treatment. The recurrence may be due to a circulating plasma factor [116]. This factor may be responsible for an increase in podocyte integrin-linked kinase activity leading to podocyte detachment from the GBM as found in some patients [41]. Recently, cardiotrophin like cytokine-1 has been identified as being a candidate for the FSGS permeability factor [115].

Lately, the role of Notch signaling, which is involved in the regulation of many cellular processes like proliferation, differentiation, and cell death [50], in the development of renal diseases is debated. Although NotchI protein plays a crucial role during kidney development, very little active NotchI can be detected in mature kidneys [134]. In glomerular epithelial cells of patients with diabetic nephropathy or FSGS, however, an increased presence of the active protein domain of NotchI was found, which, based on the homology with mouse podocyte biology [94], may be involved in apoptosis induction.

Another classification of FSGS is based on the underlying cause and subdivides it into three categories: idiopathic, genetic, and secondary due to injury, medication, or drug abuse [21, 143]. The last years genetic linkage studies have identified several genes involved in the development of FSGS and have contributed to the understanding of its pathophysiology (Table 1). A subset of genes enlisted in Table 1 are discussed below in more detail (Fig. 2).

NPHS1

One of the major components of the slit membrane is the transmembrane protein nephrin (*NPHS1*, OMIM 602716). Nephrin has an important role in maintaining the structure of the podocyte slit membrane, as shown by nephrin-deficient mice which develop proteinuria and foot process effacement [103]. Injection of anti-nephrin antibody in animals also results in foot process effacement [96]. Intracellularly, nephrin functions as a signaling molecule [46, 47]. Nephrin (and also CD2AP, another component of the slit membrane) associates with the p85 regulatory subunit of phosphoinositide 3-OH kinase (PI3K) thereby stimulating the AKT signaling pathway controlling cell growth, migration, and survival [46].

Nephrin oligomers associate with lipid rafts in the slit membrane [126]. A rat model of antibody-induced foot process effacement showed morphological changes of the filtration slits with apical dislocation and tyrosine phosphorylation of nephrin [126]. As such, tyrosine phosphorylation may regulate the subcellular redistribution of slit membrane proteins [7, 126]. Furthermore, a study in Fyn-deficient mice showed an important role for tyrosine phosphorylation in nephrin-dependent intracellular signaling [133]. Fyn, a member of the Src protein kinase family, tyrosine phosphorylates the cytoplasmic domain of nephrin and Fyn-deficient mice develop proteinuria and foot process effacement [133].

NPHS1 was found mutated in patients with the congenital nephrotic syndrome of the Finnish type (CNF, OMIM 256300). CNF has an incidence of 1:10,000 births in Finland, but less frequently in other countries. Although several missense mutations were found in CNF patients, two mutations, the Fin_{major} (deletion nucleotides 121 and 122) and Fin_{minor} (premature stop at amino acid 1109), account for over 90% of the cases in Finland [59]. Recurrence of CNF may occur in 20–25% of the patients after receiving a renal allograft and may be caused by anti-nephrin antibodies [97, 137]. In addition to the renal disease, patients with *NPHS1* mutations may also show neurological symptoms including muscular hypotonia, dyskinesia, mild cerebral atrophy, and hearing impairment of still obscure origin [70].

Table 1 Characteristics of hereditary diseases involved in nephrotic syndrome

List of diseases	Mode of inheritance	Protein	Protein function	Gene	Chrom.	Protein expression slit membrane
Congenital nephrotic syndrome of the Finnish type (CNF)	Autosomal recessive	Nephrin	Key component of the podocyte slit diaphragm	*NPHS1*	19q13.1	Podocyte slit membrane
Steroid-resistant nephrotic syndrome (SRN1)	Autosomal recessive	Podocin	Establishment of the podocyte slit diaphragm	*NPHS2*	1q25–q31	Podocyte slit membrane
Familial FSGS	Autosomal recessive	CD2-associated protein	Cytoskeletal remodeling, cell motility, endocytosis	*CD2AP*	6	Podocyte slit membrane
Familial focal segmental glomerulosclerosis (FSGS1)	Autosomal dominant	Alpha-actinin-4	Anchoring protein	*ACTN4*	19q13	Predominantly podocyte cell body
Denys-Drash syndrome (DDS)/ Frasier syndrome (FS)	*de novo* (dominant)	Wilms tumor 1	Transcription factor	*WT1*	11p13	Nucleus and cytoplasm of podocyte cell body
Familial focal segmental glomerulosclerosis (FSGS2)	Autosomal dominant	Transient receptor potential cation channel 6	Ca^{2+}entry during cell proliferation	*TRPC6*	11q21–q22	Tubules, podocytes, mesangial and endothelial cells
Early-onset familial nephrotic syndrome	Autosomal recessive	Phospholipase C epsilon	Involved in cell growth and differentiation, gene expression	*PLCE1*	10q23	Cytoplasm podocyte cell body
Pierson's syndrome	Autosomal recessive	Laminin β2 chain	Establishment of the glomerular basement membrane	*LAMB2*	3p21	Glomerular basement membrane
Mitochondrial disorder	Maternal	Non-protein tRNA	Amino acid supply	NA	mtDNA	Mitochondrion renal cells
CoQ10 deficiency	Autosomal recessive	Parahydroxy-benzoate-polyprenyl-transferase	Electron carrier in mitochondrial respiratory chain	*COQ2*	4q21.23	Mitochondrion renal cells
Nail-patella syndrome	*de novo* (dominant)	LIM homeobox transcription factor 1 beta	Transcription factor	*LMX1B*	17q11	Nucleus and cytoplasm of podocyte cell body
Schimke immuno-osseous dysplasia	Autosomal recessive	SWI/SNF2-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1	Gene regulation, replication, recombination, and DNA repair	*SMARCAL1*	2q34–q36	Nucleus of podocyte cells and proximal tubule
Mandibuloacral dysplasia	Autosomal recessive	Zinc metallo-proteinase STE24	Potentially involved in processing of farnessylated protein	*ZMPSTE24*	1p34	Unknown
Galloway-Mowat syndrome	Autosomal recessive	GMS1	unknown	*GMS1*	Unknown	Podocytes
Fechtner syndrome	Autosomal dominant	Nonmuscle myosinllA heavy chain	Actin-based motility	*MYH9*	*22q12.3*	Tubular epithelia, mesangial cells and podocyte
Action myoclonus-renal failure syndrome	Autosomal recessive	Lysosomal integral membrane protein type 2	Lysosomal degradation of macromolecules, RNA and DNA	*SCARB2*	4q13–21	Lysosomal membrane of glomerular cells (study in mice)

Chrom. chromosome, *NA* not applicable, *mtDNA* mitochondrial DNA, *GBM* glomerular basement membrane, *FP* foot process, *NS* nephrotic syndrome, *ESRD* end-stage renal disease, *FSGS* focal segmental glomerulosclerosis, *DMS* diffuse mesangial sclerosis

NPHS2

Podocin (*NPHS2*, OMIM 604766), a member of the stomatin protein family, is exclusively expressed in the podocytes and localizes at the insertion of the slit membrane. Due to its similarity to stomatin, it is believed that podocin forms a hairpin-like structure with intracellular NH_2- and COOH-termini [13, 109].

Podocin, like nephrin, associates with lipid rafts [121], and recruits nephrin and CD2AP in these rafts ensuring a stable and proper functioning filtration barrier. The COOH-terminal cytoplasmic tail of podocin interacts with nephrin and Cd2ap (the mouse homolog) [121]. This protein interaction greatly enhances nephrin-induced signaling in vitro [47]. The COOH-terminal domain of podocin also binds NEPH-1, a podocyte slit membrane protein structurally related to nephrin [122]. NEPH-1 is involved in maintaining the structure of the filtration barrier and also interacts with nephrin [75].

Podocin dysfunction leads to alterations of the slit membrane assembly and to proteinuria in experimental models. $NPHS2^{-/-}$ mice develop proteinuria and massive mesangial sclerosis (different from FSGS seen in humans), the podocytes are enlarged and focally vacuolized. The sclerosis rapidly progresses with age. Beside the absence of podocin, no nephrin is found in the foot processes as well. The podocin-deficient mice die a few days after birth [110].

In human, *NPHS2* mutations, are mainly associated with autosomal recessive steroid-resistant nephrotic syndrome (SRN1, OMIM 600995) [70], but were also found in sporadic cases of steroid-resistant nephrotic syndrome patients [16, 57, 67, 132]. A recent study has demonstrated that also milder *NPHS1* mutations can cause a childhood-onset steroid-resistant nephrotic syndrome with underlying histological lesions ranging from minimal change nephropathy to FSGS [101].

The podocin variant R229Q is often found and has an allele frequency of 3.6% in a control population. The mutant protein has a decreased binding efficiency to nephrin and enhances FSGS susceptibility in association with a second *NPHS2* mutation [132]. The R229Q variant is also associated with microalbuminuria in the general population [100].

Mutated podocin may cause a disturbance in recruiting nephrin to the plasma membrane [49, 111], which is comparable with the lack of nephrin in the slit membrane found in podocin-deficient mice [110]. The R138Q NPHS2 mutant (one of the most common found mutation) results in retainment of the mutant podocin in the endoplasmatic reticulum. The R138X NPHS2 mutant yields a mutant podocin protein that is not able to associate with lipid rafts in the plasma membrane. Both mutant proteins were unable to recruit nephrin to the lipid rafts and lost their ability to enhance nephrin signaling [49, 111]. NH_2-terminal mutant proteins were still able to associate with lipid rafts and had no effect on nephrin localization [95]. Collected clinical data from patients with *NPHS2* mutations showed that podocin mutants retained in the endoplasmatic reticulum are associated with earlier onset of the disease than those correctly targeted to the cell membrane [111].

Early reports showed that patients with *NPHS2* mutations had no recurrence of FSGS after renal transplantation. Now it is believed that patients with *NPHS2* mutations have a lower risk for recurrent FSGS after renal transplantation compared to patients with idiopathic FSGS [10, 112, 139]. Patients carrying a heterozygous *NPHS2* mutation show a higher risk for recurrent FSGS (five out of eight published) in contrast to patients with homozygous or compound heterozygous mutations (five out of 68 published) [17]. The authors state that grafts from carriers of *NPHS2* mutations, such as from parents, should be avoided because of the higher risk of recurrent FSGS. Furthermore, carriers of heterozygous *NPHS2* mutations should be strictly monitored in the post-graft phase [17].

CD2AP

Although first found in a yeast two-hybrid screen as a protein binding to the T-cell membrane protein CD2 during cell–cell interaction [28], an important role for Cd2ap (CD2-associated protein) in the kidney became evident when Cd2ap knockout mice died because of renal failure involving the glomerulus [125]. The $Cd2ap^{-/-}$ mice died at an age of 6 to 7 weeks, but already at 1 week glomeruli were increased in size and cellularity (mesangial cell proliferation and glomerulosclerosis) and electron microscopy showed extensive foot processes effacement [125]. $Cd2ap^{+/-}$ mice showed no proteinuria, but have an increased susceptibility to glomerular injury by immune complexes and nephrotoxic antibodies [60]. At 9 months of age, glomerular lesions were present with an increase in mesangial cellularity. Some lesions were similar to human FSGS and for this reason primary FSGS patients were tested for mutations in the *CD2AP* gene (the human homolog of Cd2ap, originally named CMS, OMIM 604241) [60] One heterozygous splice-site mutation was detected in two patients. The predicted mutated protein would lack more than 80% of the protein. Immunoblotting CD2AP isolated from immortalized B-lymphocytes showed a reduction in CD2AP expression in these patients [60]. Beside the heterozygous splice-site mutation one homozygous *CD2AP* mutation has been described [77]. This mutation results in a premature stop codon and a protein truncation of 4% at the COOH-terminus. No CD2AP protein was found in the patients' lymphocytes. Both

parents, who are heterozygous for the mutation, show no kidney pathology and no decrease in CD2AP expression in lymphocytes. These two different reports show that not all heterozygous *CD2AP* mutations cause kidney disease. The development of FSGS clearly depends on the severity of the mutation [77].

CD2AP/Cd2ap is a multifunctional adaptor molecule localized to the cytoplasm, membrane ruffles, and leading edges of cells [61]. The protein plays a role in cytoskeletal remodeling [28, 61], cell survival [46, 118], and endocytosis [19, 63, 80]. The COOH-terminus of CD2AP directly interacts with the cytoskeletal protein filamentous actin (F-actin) [61, 71] and synaptopodin, an actin-bundling protein [48]. At the slit membrane, CD2AP interacts with nephrin and podocin [121, 124] and serves as a linker anchoring slit membrane proteins to the actin cytoskeleton of podocytes.

CD2AP and nephrin bind to the p85 regulatory subunit of phosphoinositide 3-OH kinase (PI3K) stimulating the serine–threonine kinase AKT [46]. One of the target proteins of AKT is Bad, a proapoptotic Bcl2 family member that interacts with prosurvival Bcl2 family members to promote apoptosis. Phosphorylated Bad is inactive and protects podocytes against detachment-induced cell death [46].

Combined mutations in two podocyte genes may be a common etiology for glomerular disease [48]. Crossbreeding heterozygous Cd2ap knockout mice with mice heterozygous (knockout) for synaptopodin or Fyn, which alone did not result in clinical kidney pathology, resulted in spontaneous proteinuria and FSGS-like glomerular damage supporting a role for CD2AP haploinsufficiency [60]. A role of combined mutations in the development of glomerular disease is also seen in a patient group with non-familial childhood-onset FSGS. One patient showed a *CD2AP* mutation combined with a heterozygous *NPHS2* mutation [76].

ACTN4

Alpha-actinin-4 (ACTN4, OMIM 604638) is an actin-bundling protein important for the integrity of the podocyte cytoskeleton associated with cell motility. Transgenic ACTN4 mice develop a severe glomerular disease and FSGS [66, 86]. Lymphocytes of homozygous ACTN4-deficient mice displayed an increase in lymphocyte chemotaxis, supporting the role of ACTN4 in cell motility [66]. Podocyte cell lines derived from these ACTN4-deficient mice show less adherence to the GBM components collagen IV and laminins-10 and laminins-11, also indicating its role in maintaining glomerular architecture and preventing disease [24].

ACTN4 is highly expressed in several human tissues. Despite the overall expression of ACTN4, the human phenotype associated with *ACTN4* mutations only manifests in the kidney. Mutations in *ACTN4* are associated with an autosomal dominant form of familial FSGS (OMIM 603278). By now, only three *ACTN4* missense mutations have been described in literature [55, 140]. The mutated ACTN4 proteins showed a higher binding affinity to F-actin, and may change the mechanical characteristics of the podocyte [55]. In vitro studies in podocytes of mice homozygous for the human disease-causing K225E substitution, shows aggregation of the mutant α-actinin-4 with F-actin. Furthermore, the conformational change also leads to an increase of the actin-binding stoichiometry and a resistance to regulation by Ca^{2+} [141]. In sporadic cases of FSGS, without an apparent familial disease pattern, ACTN4 amino acid substitutions were also found [140]. However, cellular localization of the mutated proteins and actin-binding assays show that these mutations are probably not disease-causing or contributing to the disease [140].

WT1

Another well-described genetic defect in patients with primary nephrotic syndrome is the spectrum of clinical pictures caused by mutations in WT1 (for review 74, 90, 135, OMIM 607102), a transcription factor regulating several programs of cellular proliferation and differentiation. The *WT1* gene is positionally cloned based on its role in the development of Wilms tumors (OMIM 194070), the most common form of cancer in children [33, 38]. Heterozygous de novo mutations in *WT1* cause Denys-Drash syndrome (DDS, OMIM 194080) and Frasier syndrome (FS, OMIM 136680), two overlapping syndromes [84], characterized by nephrotic syndrome with either diffuse mesangial sclerosis (DMS in DDS) or FSGS (in FS), genitourinary defects, and a higher risk of developing Wilms tumor (in DDS) or gonadal dysgerminoma (in both DDS and FS).

WT1 has a transcription-regulating domain (encoded by exons 1 to 6) and a DNA-binding zinc finger domain (encoded by exons 7 to 10). There are four major isoforms due to the insertion of the amino acids KTS between zinc fingers 3 and 4 (alternative splice site directly situated after exon 9), and due to the insertion of 17 amino acids completely encoded by exon 5. The insertion/deletion of KTS influences the space between zinc fingers 3 and 4 and thereby the binding properties of the protein [39].

The mutations found in DDS patients, but also in cases of isolated DMS, are mostly missense mutations (80%) in zinc fingers 2 and 3 (exon 8 and 9). The R394W (1180 C>T, exon 9) substitution is most frequently found in almost 50% of the DDS patients [14, 74, 99]. These

mutations affect the DNA-binding stability of WT1 to the target gene [73]. FS patients usually present with intron 9 splice-site mutations affecting the +KTS/−KTS ratio [6, 62]. The fact that DDS and FS are overlapping syndromes is strengthened by the finding of intron 9 splice-site mutations in patients with a phenotype of DDS [25, 39, 53, 84], and an exon 9 mutation in a patient with FS [64].

TRPC6

Transient receptor potential cation channel subfamily C, 1-7 (TRPC) is a subgroup of the TRP family of cation channels involved in the regulation of Ca^{2+} influx. These ion channels can be activated subsequent to either depletion of Ca^{2+} from internal stores or through receptor-mediated processes [107]. TRPC channels are expressed in many tissues. In the podocytes TRPC-1, TRPC-2, TRPC-5, and TRPC-6 are expressed [106]. TRPC6 (OMIM 603652) is localized to the podocyte cell body, primary processes and in close vicinity to the slit membrane were it interacts with nephrin and podocin (not CD2AP) [106]. TRPC6 is also abundantly expressed in mesangial cells [127]. In diabetic nephropathy, the mesangial contractile function is impaired, most probably due to a reduced Ca^{2+} influx. A recent study showed that high glucose downregulates the TRPC6 protein which might contribute to the impaired Ca^{2+} signaling of mesangial cells seen in diabetes [36].

TRPC6 was found mutated in families with an autosomal dominant form of FSGS (OMIM 603965) [106, 142]. These mutations may cause a gain of function. The P112Q mutated TRPC6 protein showed an enhanced influx of Ca^{2+}, especially after activation of the G-protein-coupled receptor AT1 by angiotensin II, and the cellular localization of the mutant protein is more situated at the plasma membrane [142]. An increase in Ca^{2+} influx is also seen in two other missense mutations (R895C and E897K), but absent in three different ones (N143S, S270T, and K874X) [106]. In these cases an altered channel regulation or an altered interaction with other slit membrane proteins (like nephrin and podocin) may cause the disease [106]. In addition to the effects of gain-of-function mutations in the *TRPC6* gene, also elevated levels of wild-type TRPC6 protein in some acquired glomerular diseases (like membranous nephropathy and puromycin aminonucleoside-induced albuminuria) may lead to podocyte dysfunction [88]. The pathways that TRPC6 may modulate are discussed in a recent seminar [119].

PLCE1

PLCE1 (phospholipase C epsilon, OMIM 608414) belongs to the phospholipase C (PLC) family involved in intracellular signaling, necessary for cell growth and differentiation. PLC plays an important role in regulating the Ca^{2+} release from internal stores as well as the influx of Ca^{2+} through cation channels (like TRPC). PLC is activated by binding of a hormone or growth factor to its receptor and PLC in turn converts phosphatidylinositol-4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. Plasma membrane channels are activated and cations flow into the cell [31].

In the kidney, PLCE1 expression is found in the podocyte cell body and foot processes [43]. PLCE1 expression appears at the S-shaped stage of glomerular development and is highly expressed during the early capillary loop stage [43]. Recently, positional cloning identified the *PLCE1* gene being involved in families with early-onset NS and ESRD (OMIM 610725) [43]. Renal histopathology generally shows DMS and histochemistry reveals reduced nephrin expression. In two patients (carrying the nontruncating missense mutation S1484L) biopsies revealed FSGS. In these patients the age of onset was relatively late as was the age of reaching ESRD [43]. A recent study in patients with isolated (non-syndromic) DMS, showed that *PLCE1* truncating mutations are more frequently found than mutations in *WT1* or *LAMB2*, two genes known to cause isolated DMS as well [32]. The authors speculate that missense mutations in *PLCE1* may be associated with a milder disease course in isolated DMS or other histologic variants such as FSGS [32, 43].

A zebrafish knockdown model was used to investigate the role of PLCE1 in the maintenance of the podocyte filtration barrier during development. The zebrafish *PLCE1* ortholog was knocked-down in embryo's using antisense morpholino oligonucleotides and barrier function was assayed at 4-day-old embryos by vascular retention of a large FITC-labeled tracer molecule. In control embryos, the tracer molecule retained in the vasculature. Morpholino-injected embryos showed abundant FITC-positive endocytic vesicles in the pronephric tubule, distal to the glomerulus. This indicated a breakdown of the barrier function in the pronephric glomerulus [43].

Laminin β-2

The heterotrimeric laminin is assembled by three polypeptide chains: α, β, and γ. Different isoforms have been identified. Laminin-11 (α5β2γ1) is predominantly found in the adult GBM and replaces the laminin-1 (α1β1γ1) isoform, initially expressed during kidney development [129].

Mutations in the *LAMB2* gene, encoding the laminin β2 chain (OMIM 150325), are associated with the Pierson's syndrome (OMIM 609049). This syndrome is characterized by early-onset nephrotic syndrome with DMS rapidly

progressing to ESRD and distinct ocular abnormalities (in particular microcoria) [146]. The *LAMB2* mutations result in loss of laminin-β2 expression in the kidney and other tissues studied [146]. Mutations were also found in patients with congenital nephrotic syndrome (and FSGS) with or without minor ocular changes [40]. These results indicate genotype–phenotype correlations among patients with *LAMB2* mutations. The authors speculated that complete loss-of-function (e.g. truncating) mutations appear to be associated with the complete Pierson syndrome, whereas missense mutations may display variable phenotypes ranging from a milder variant of Pierson's syndrome to isolated congenital nephrotic syndrome with or without minor ocular abnormalities [40]. The frequency of hematuria in these patients is not clear from the clinical data.

Mitochondrial disorders

Mitochondrial cytopathies, either caused by mutations in the maternally inherited mitochondrial DNA (mtDNA) or in nuclear DNA, represent a heterogeneous group of multisystem disorders [120]. Mitochondria are non-uniformly distributed in tissues and mutated and wild-type mtDNA coexist in cells (heteroplasmy). These characteristics contribute to the large variety of clinical symptoms seen in mtDNA mutations [120].

Mutations in the $tRNA^{Leu(UUR)}$ (OMIM 590050) gene are mainly associated with the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, OMIM 540000) [102]. The most common transition A3243G is also found in patients with FSGS sometimes associated with maternally inherited diabetes and/or sensorineural hearing loss [18, 27, 37, 44, 52, 69, 92, 144]. In most patients proteinuria is below nephrotic range and the FSGS progresses slowly.

The effect of the A3243G mutation to the function of the $tRNA^{Leu}$ has been studied [145]. A3243G mutant $tRNA^{Leu}$ have a shorter life span and the extent of aminoacylation ("charging" of a tRNA with an amino acid) was rather low (less than 30%) compared to wildtype [145].

Mutations were also found in other mitochondrial tRNA genes. In one patient, a A5843G transition was found in the $tRNA^{Tyr}$ (OMIM 590100) gene. The patient presented with mitochondrial cytopathy preceded by steroid-resistant FSGS. A skeletal muscle biopsy showed a combined respiratory chain deficiency and a partial deficiency of coenzyme Q10 most probably secondary to the oxidative damage [117]. Finally, the A4269G substitution in the $tRNA^{Ile}$ gene (OMIM 590045) is described in a patient with mitochondrial encephalomyopathy and multi-organ disorders including deafness, epilepsy, FSGS, and dilated myopathy later in life [130].

Coenzyme Q10 (CoQ10) plays an important role in the electron transport from complex I and II to complex III of the mitochondrial respiratory chain. Coenzyme CoQ10 deficiency (OMIM 607426) is associated with a variety of clinical phenotypes. Recently, a mutation in the nuclear DNA was found in a patient with CoQ10 deficiency [104]. This patient presented at age 12 months with proteinuria due to FSGS, mild psychomotor delay, and optic atrophy. Homozygosity mapping revealed a homozygous mutation in the *COQ2* gene, encoding *para*-hydroxybenzoate-polyprenyl-transferase (OMIM 609825), an enzyme involved in the CoQ10 biosynthetic pathway [104]. The pathogenity of the mutation was demonstrated by complementation experiments in COQ2-deficient yeast and showed full functional complementation after transformation with wild-type COQ2 but not mutant COQ2 [78]. This genetic defect of the respiratory chain, is related to late-onset nephrotic syndrome with multiple organ involvement. However, respiratory chain deficiency has also been reported in one infant with congenital nephrotic syndrome characterized by diffuse mesangial hypercellularity and focal tubular dilation [35] and in one infant with glomerular lesions characterized by crescentic glomerulonephritis [26]. The encephalopathy in patients with primary CoQ10 deficiency may be improved by oral CoQ10 supplementation. Recently, it is shown that early administration of CoQ10 may also result in progressive recovery of the renal function and reduction of proteinuria [89].

Other rare cases of hereditary FSGS and recent findings

The nail-patella syndrome (OMIM 161200) is a rare autosomal dominant disorder characterized by dysplasia of the nails, absent or malformed patellas, dysplasia of the elbows and frequently glaucoma and progressive nephropathy. Renal biopsies reveal non-specific findings mostly related to the degree of renal failure, including FSGS, proliferative glomerulonephritis with crescent formation, and hyalinization of the glomeruli [12]. The gene involved is the transcription factor *LMX1B* (OMIM 602575) which, among others, is required for expression of CD2AP protein and podocin [87].

Mutations in *SMARCAL1* (OMIM 606622) are involved in the development of Schimke immuno-osseous dysplasia (OMIM 242900). This autosomal recessive disorder is characterized by spondyloepiphyseal dysplasia causing growth retardation, defective cellular immunity, hyperpigmented macules, dysmorphic facial feature, and nephrotic syndrome (due to FSGS). *SMARCAL1* encodes an actin-dependent regulator of chromatin which is involved in gene regulation, replication, recombination, and DNA repair [11]. More detailed studies of its role are

provided by Elizondo et al. [29]. Recently, mutations in *SMARCAL1* were also found in two siblings with an incomplete phenotype of Schimke immuno-osseous dysplasia. The siblings were initially classified as suffering from familial steroid-resistant nephrotic syndrome. In prepuberty they had proportionate short stature which developed into disproportions during adolescence. No other syndrome-specific symptoms were found. Milder phenotypes may be clinically overlooked [147]. It has been demonstrated that anthropometric measures are helpful to distinguish Schimke immuno-osseous dysplasia from other forms of chronic kidney disease [79].

In three patients (of two families) a nonsense mutation in tetraspanin *CD151* (OMIM 602243) causes end-stage familial nephropathy with pretibial epidermolysis bullosa and sensorineural deafness (OMIM 609057). The only available renal biopsy of one patient did not show FSGS but splitting of the tubular basement membrane and thickening and fragmentation of the GBM [56]. CD151 null mice, however, develop massive proteinuria while aging caused by FSGS [113].

Mandibuloacral dysplasia (MAD, OMIM 248370/608612) is a rare autosomal recessive syndrome with variable clinical features. These features include mandibular and clavicular hypoplasia, acro-osteolysis of terminal phalanges, delayed closure of cranial sutures, joint contractures, mottled pigmentation, and lipodystrophy. MAD is also genetically heterogeneous: two loci have been identified. So far, most mutations were found in *LMNA*, encoding lamin A (OMIM 150330), a structural protein component of the nuclear lamina determining nuclear shape and size [51]. Only three patients have been reported with compound heterozygous mutations in *ZMPSTE24* (OMIM 606480). This gene encodes a zinc metallo-proteinase involved in post-translational processing of prelamin A. Two of them presented with renal failure caused by FSGS, suggesting FSGS as a phenotypic manifestation in patients with ZMPSTE24 deficiency [1].

The Galloway-Mowat syndrome (GMS, OMIM 138770) is an autosomal recessive disorder characterized by microcephaly, severe mental retardation, hiatal hernia, and steroid-resistant nephrotic syndrome. In GMS, the nephrotic syndrome occurs in the first 4 months of life, is steroid-resistant and rapidly progresses to end-stage renal disease. Histology shows heterogeneous lesions: minimal changes, endocapillary proliferation, FSGS (in most cases), and, in end stages, DMS [114]. Recent linkage studies in two Algerian families identified a homozygous mutation in the *GMS1* gene. The protein encoded by this gene is expressed in many tissues, including brain and glomerular podocytes, and has yet an unknown function [148].

Recently, *MYH9* and *SCARB2* have been identified as being involved in the development of FSGS. *MYH9* encodes for the heavy chain of nonmuscle myosinIIA (NMMHC-IIA, OMIM 160775). Myosins of class II are widely distributed in most tissues and are essential components of the cell motor system involved in several important cell functions. These, among others, include phagocytosis, maintenance of cell shape and polarity, and intracellular organelle/particle trafficking [81]. Nonmuscle myosinII is composed of two heavy chains and two pairs of light chains. The NH_2-terminal region contains an actin and ATP-binding domain required for motor activity. The COOH-terminus allows the molecules to form filaments [123]. In the kidney, NMMHC-IIA is localized in the tubular epithelia and in the glomeruli it is expressed in podocytes and mesangial cells [34].

Heterozygous mutations of *MYH9* are involved in a complex disorder named *MYH9*-related disease characterized by platelet macrocytosis, thrombocytopenia, and leukocyte inclusions containing NMMHC-IIA. Complications that may arise are deafness, cataracts, and renal failure. In the past, these patients were classified as being affected by May–Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, or Epstein syndrome (OMIM 155100, 605249, 153640, and 153650, respectively). It is now believed that these disorders are not distinct entities, but rather a single illness with a continuous clinical spectrum [81]. In a large family with Fechtner syndrome, ten members carried a *MYH9* missense mutation. Only two members had renal problems. Electron microscopy showed FSGS and segmental effacement of podocytes in these patients [34]. Why the other family members did not develop renal problems remains unclear. Most probably other additional factors play a role [34, 65]. More recently, *MYH9* was suggested to be a risk gene for the development of idiopathic FSGS by usage of a genome scan on African–American individuals with FSGS. However, sequencing of the 40 exons and intron–exon junctions of MYH9 did not reveal any causal sequence variation. The authors hypothesize that the variation may occur in regulatory elements or splice-site determinants influencing RNA expression or protein structure [65]. Future studies are necessary to identify the precise role of *MYH9* in the development of FSGS.

Action myoclonus-renal failure (AMRF, OMIM 254900) syndrome is a lethal inherited form of progressive myoclonus epilepsy associated with renal failure due to FSGS. It usually starts at 15–25 years of age with proteinuria or neurological symptoms as tremor, action myoclonus, or seizures associated with storage material in the brain [9]. Mutations in *SCARB2* (OMIM 602257) were found in three families with a single AMRF proband [5, 9]. However, analysis of patients with non-syndromic FSGS, MCNS, thin-basement membrane nephropathy, or non-Alport with hematuria and proteinuria did not reveal any mutations

indicating a specific role of *SCARB2* in AMRF [9]. *SCARB2* encodes for the ubiquitously expressed lysosomal integral membrane protein type 2 (LIMP-2) mainly found in lysosomes and late endosomes [5]. This LIMP-2 protein has been shown to act as a receptor to bind β-glucocerebrosidase, the enzyme defective in Gaucher disease (lysosomal storage disorder) [105]. LIMP-2 deficient mice have also a kidney phenotype. The kidneys, which are non-hydronephrotic show pelvi-ureteric obstruction and glomerular lesions with mesangial hypercellularity and effacement of foot processes [9].

Conclusion

In the last years, knowledge of the genetic defects described above provided new insight in the structure and function of the podocyte slit membrane and the development of nephrotic syndrome. FSGS patients are a heterogeneous group with different underlying causes. Although the development of FSGS is not completely and solely explained by the presence of genetic abnormalities, the knowledge whether a FSGS patient carries a genetic defect turns out to be very important for determining treatment strategy and prognosis of the individual patient. For example, most of the patients with *NPHS2* mutations are steroid-resistant and therefore treatment with steroids may be discontinued after obtaining the results of DNA analysis. Furthermore, in most cases, there is no recurrence of nephrotic syndrome after renal transplantation in patients with *NPHS2* mutations. In patients carrying *WT1* mutations further medical examination and regular check-ups are required given the higher risk of developing malignancies. Mitochondrial DNA mutations may cause multi-systemic disorders requiring regular neurological follow-up and glycemia control. Finally, combined genetic defects in podocyte genes may play a role in the development of FSGS.

How to approach a patient with steroid-resistant nephrotic syndrome with FSGS in the biopsy? FSGS is not a specific histopathologic lesion. Similar alterations may be observed in a lot of other disorders [93] which should be excluded. When a hereditary disorder is suspected (a familial occurrence is not required) careful clinical and biochemical investigations could direct the research (neurologic symptoms, bone, eye, ear, genital abnormalities, thrombocytopenia). As the next step, the analysis can start with screening for the most frequent genetic disorders, taking the date of the first manifestation of the nephrotic syndrome in consideration. However, this frequency depends on the country of origin. It is not known for all genes. For *NPHS2* an extensive evaluation was made by Berdeli et al. [15]. The incidence of podocin mutations in familial steroid-resistant nephrotic syndrome was found to be 29.2% in Turkey, being 40% in European and American children, and 0% in Japanese and Korean children. The incidence of sporadic podocin mutations in Turkey (24%) was similar to that in European and American children (10–30%), but higher than in Chinese (4%) and Japanese and Korean children (0%). *NPHS1* mutations are frequent in Finland. Two thirds of cases of nephrotic syndrome in the first year of life in a large European cohort are caused by mutations in four genes (*NPSH1*, *NPSH2*, *WT1*, and *LAMB2*) [42].

Further studies of the numerous podocyte genes coming from human and animal studies will undoubtfully identify new players on the edge of glomerular filtration barrier and will provide new insights into the pathogenesis of nephrotic syndromes in humans. The insight in the podocyte function obtained from the study of these genetic disorders will help to explain the effect of medication used in the treatment of glomerular disorders. Most drugs used in the treatment of nephrotic syndrome have a direct effect on the podocytes (corticoids [136], inhibitors of angiotensin-converting enzyme [45], COX2 inhibition [138], mizoribine [91], and cyclosporine [30]). The antiproteinuric effect of cyclosporine attributed to its immunosuppressive action, may also result from stabilization of the actin cytoskeleton in the podocyte. Cyclosporine protects synaptopodin from cathepsin l-mediated degradation. Synaptopodin is an important regulator of podocyte function (see Fig. 2) [82]. Recent studies in the action of cyclosporine, frequently used in the treatment of FSGS [85], highlight this new concept [30].

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