CHAPTER 13

Hereditary Nephrotic Syndrome

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During the past decade, defects in various genes have been associated with the development of steroid-resistant nephrotic syndrome (SRNS) in children and adults. These genes encode for proteins that participate in the development and structural architecture of glomerular visceral epithelial cells (podocytes). This novel insight moved the podocyte with its interdigitating foot processes and slit diaphragms (SD) into the center of interest regarding the pathophysiology of proteinuria.

Whereas light microscopy shows variable aspects ranging from minimal change nephropathy to diffuse mesangial sclerosis or focal and segmental glomerulosclerosis (FSGS) (Figure 13-1), all hereditary proteinuria syndromes share a common phenotype when evaluated by electron microscopy, which uniformly demonstrates the typical flattening of the foot processes and loss of the SD. With respect to the clinical course, two entities can be distinguished: disorders of early glomerular development manifesting prenatally, directly after birth, or in early infancy, and disorders with late-onset nephrotic syndrome, typically manifesting as FSGS in adulthood. In the following sections, important genes involved in both types of manifestations are discussed.

HEREDITARY DISORDERS OF EARLY GLOMERULAR DEVELOPMENT

Podocytes develop from the nephrogenic blastema in a chain of events in conjunction with development of the renal glomeruli. First, local condensation of the mesenchyme leads to the formation of the nephron anlage, that is, the comma-shaped and the S-shaped bodies, and eventually the formation of the mature glomerulus. Podocytes are the first cells that can clearly be distinguished in this process, forming a disklike layer of epithelial cells. The subsequent differentiation to mature podocytes with interdigitating primary and secondary foot processes is associated with a general loss of ability for further proliferation. At this stage, early cell-cell contacts (adherens junctions) have developed into a specialized structure, the SD, spanning the intercellular space. The final glomerular filtration barrier is constituted by the fenestrated endothelium, the glomerular basement membrane (GBM), and interdigitating podocytes.

A number of genes are involved in these processes (Table 13-1; Figure 13-2), and *WT1* is one of the major mediators of podocyte differentation. *NPHS1* and *NPHS2* code for nephrin and podocin, respectively, two proteins that have important roles for the organization of the SD. *LAMB2* encodes laminin β2, one component of the heterotrimeric laminins that link the podocyte to the GBM. *LMX1B* encodes the transcription factor Lmx1b that in the kidney is exclusively expressed in podocytes. It is one of the crucial genes regulating gene expression during early steps of podocyte development. The most recently identified gene involved in early-onset nephrotic syndrome *NPHS3/PLCE1*, encodes for phospholipase C epsilon-1, involved in podocyte signaling processes.

WT1 Gene Mutations

Wilms' tumor is one of the most common solid tumors of childhood, occurring in 1 of 10,000 children and accounting for 8% of childhood cancers. The Wilms' tumor suppressor gene *(WT1)* was first identified in 1990.[1] *WT1* locates on chromosome 11p13 and encodes a zinc finger transcription factor that regulates the expression of many genes during kidney and urogenital development. Mutations in *WT1* were first identified in pediatric patients affected by Wilms' tumor, aniridia, genitourinary malformations, and mental retardation (WAGR syndrome).[2] These were truncating mutations, associated with complete loss of function of WT1. *WT1* mutations were also identified in patients with isolated Wilms' tumor.[3] In tumor material of isolated cases, both germline and somatic mutations have been detected. Familial Wilms' tumor forms seem to follow a dominant pattern of inheritance, with dominant germline mutations. However, in a number of these cases the classical two-hit inactivation model, with loss of heterozygosity due to a second somatic event, has been described as the underlying cause of tumor development.[4]

Subsequently, *WT1* mutations were also associated with Denys-Drash syndrome (DDS),[5] Frasier syndrome (FS),[6] and diffuse mesangial sclerosis (DMS) with isolated nephrotic syndrome (NS) (Jeanpierre et al., 1998).[7] The full picture of autosomal dominant Denys-Drash syndrome is characterized by early-onset NS, male pseudohermaphroditism, gonadal dysgenesis, and development of Wilms' tumor (in more than 90% of patients). Wilms' tumor may precede or develop after the manifestation of NS. Age at onset of NS is generally within the first months of life.[8] In rare cases enlarged and hyperechogenic kidneys are already demonstrated by prenatal ultrasound.[9] Renal histology typically presents with DMS[10] and electron microsocopy reveals foot process effacement.

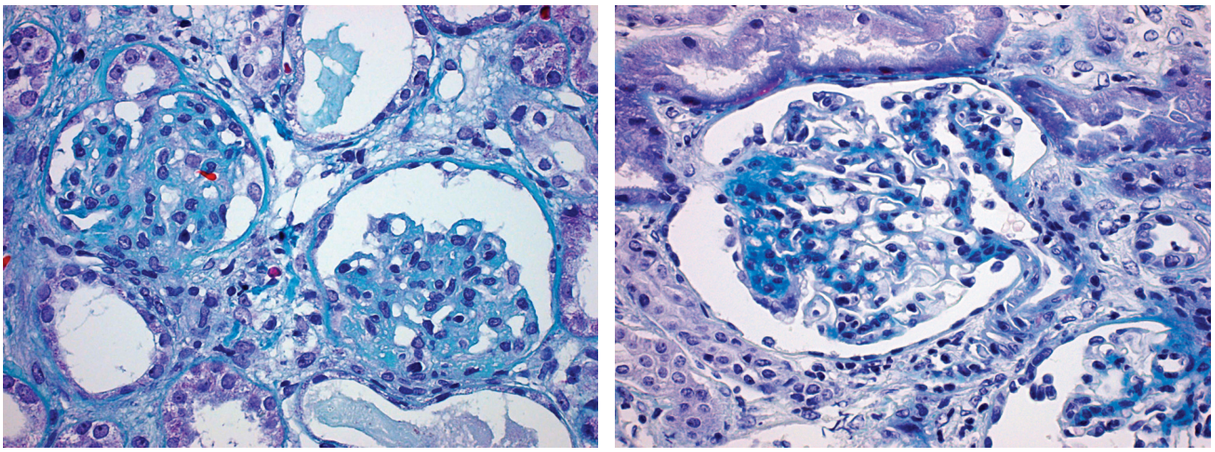

Figure 13-1 Kidney histology of a patient with diffuse mesangial sclerosis **(A)** and focal segmental glomerulosclerosis **(B),** respectively. (Courtesy Rüdiger Waldherr, Praxis für Pathologie, Heidelberg, Germany.)

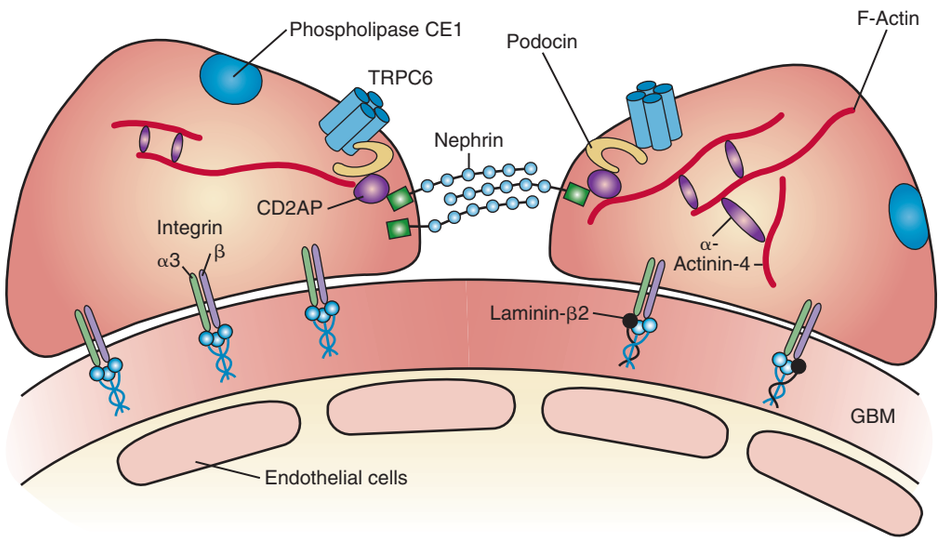

Figure 13-2 Schema of a podocyte foot process cross section depicting important components involved in hereditary nephrotic syndrome.

The NS is resistant to steroid treatment and renal function deteriorates rapidly to end-stage renal disease (ESRD) during infancy. Bilateral nephrectomy is generally advised in ESRD to prevent development of Wilms' tumor.[11] Recurrence of NS after kidney transplantation has not been observed so far.[12]

Dominant *WT1* mutations are identified in the vast majority of DDS patients. These mutations predominantly affect exons 8 and 9 of the *WT1* gene and most of them are de novo mutations not observed in the parents. Most *WT1* mutations associated with DDS are missense mutations affecting conserved amino acids of the zinc finger domains, with R394W being the most frequent mutation observed. These alterations of the zinc finger structure reduce the DNA-binding capacity of the WT1 protein.[13] A heterozygous knock-in mouse model has been created for the R394W missense mutation, presenting with DMS and male genital anomalies[14] supporting the dominant nature of the disease.

Of note is that some patients affected by *WT1* mutations in exons 8 and 9 do not display with the full picture of DDS but rather with isolated DMS. *WT1* analysis should therefore be performed in all children with isolated DMS and early-onset NS because of the high risk of Wilms' tumor development in case of a positive mutation analysis result. Close monitoring by renal ultrasound (e.g., every 6 months) is important in all children with *WT1* mutation and early-onset NS. In addition, karyotype analysis is recommended in all girls with isolated DMS to detect a possible male pseudohermaphroditism. Some patients with isolated DMS display with recessive mutations in *WT1*, with both the maternal and paternal allele being affected.[7]

TABLE 13-1 **Overview on Important Disorders Causing Hereditary Nephrotic Syndromes**

	Inheritance	Locus	Gene	Protein	OMIM Accession No.
Early-onset nephrotic syndrome					
Isolated DMS	AR	11p13	*WT1*	WT1	256370
Denys-Drash syndrome (typically DMS)	AD	11p13	*WT1*	WT1	194080
Frasier syndrome (typically FSGS)	AD	11p13	*WT1*	WT1	136680
Congenital nephrotic syndrome/Finnish type	AR	19q13	*NPHS1*	Nephrin	602716
Recessive familial SRNS	AR	1q25	*NPHS2*	Podocin	600995
Recessive nephrotic syndrome	AR	10q23-q24	*NPHS3/PLCE1*	PLCE1	608414
Pierson syndrome	AR	3p21	*LAMB2*	Laminin β2	609049
Nail-patella syndrome	AD	9q34.1	*LMX1B*	Lmx1b	161200
Recessive SRNS with sensorineural deafness	AR	14q24.2	*?*	?	(to follow)
Late-onset nephrotic syndrome/FSGS					
FSGS1	AD	19q13	*ACTN4*	α-Actinin 4	603278
FSGS2	AD	11q21-22	*TRPC6*	TRPC6	603965
FSGS3 (CD2AP-associated disease susceptibility)	AD	6	*CD2AP*	CD2AP	607832

AD, Autosomal dominant; *AR*, autosomal recessive; *?*, unknown.

Frasier syndrome is characterized by a progressive glomerulopathy and male pseudohermaphroditism also[15]; however, differences from DDS include later onset of proteinuria in childhood and slower deterioration of renal function. ESRD develops only in the second or third decade of life. As in DDS, proteinuria and NS are steroid resistant. Renal histology in FS patients typically shows FSGS[16]; in a minority of patients only minimal change lesions are observed. In female patients the genitourinary tract is normally developed, whereas a complete sex reversal with gonadal dysgenesis is observed in 46,XY patients. Primary amenorrhea in conjunction with NS is a typical feature of these 46,XY patients and should prompt molecular analysis of *WT1*. Although the risk of developing Wilms' tumor is low in patients with FS, gonadoblastomata developing from gonadal dysgenesis are frequently observed. After diagnosis of FS, gonadectomy is highly recommended in 46,XY patients.

In 1997 it was first demonstrated that mutations in the *WT1* gene also underly the pathogenesis of FS.[6] Notably, the class of mutations in FS differs from DDS: whereas mutations affecting the coding sequence of exons 8 and 9 cause DDS, mutations associated with FS represent donor splice-site mutations located in intron 9. Similar to DDS, these mutations occur in a heterozygous state and frequently are de novo mutations not observed in the parents. The donor splice site of intron 9 plays an important role for the generation of the KTS isoform of the WT1 protein. This isoform contains three additional amino acids (lysine-threonine-serine; KTS). It has been demonstrated that the (+) KTS/(–) KTS protein dose ratio is of high relevance for WT1 action during genitourinary and kidney development. In FS patients this ratio is markedly reduced due to the splice-site mutations.[6]

Although the pathogenicity of *WT1* mutations is beyond any doubt, there is remarkable phenotypical heterogeneity: splice-site mutations typical for FS may in some cases be found in patients with DDS[5] or isolated DMS,[17]and patients with typical DDS mutations may display with isolated FSGS[18] or Wilms' tumor without NS.[19]

NPHS1 Gene Mutations Associated with Autosomal Recessive Congenital Nephrotic Syndrome of the Finnish Type

Congenital nephrotic syndrome (CNS) of the Finnish type (CNF) is characterized by autosomal recessive inheritance and development of proteinuria in utero.[20] The responsible gene was mapped in 1994 to chromosome 19q13[21] and mutations in *NPHS1* have been subsequently identified in affected children.[22] *NPHS1* encodes for nephrin, a zipperlike protein of the glomerular SD. Typically, severe NS manifests before 3 months of age, and renal biopsy specimens show immature glomeruli, mesangial cell hypercellularity, glomerular foot process effacement, and pseudocystic dilations of the proximal tubules. NS is steroid resistant in these patients, and treatment options include albumin infusions, pharmacological interventions with ACE inhibitors and indomethacin, and ultimately unilateral or bilateral nephrectomy.[23-26]

Nephrin is exclusively expressed in podocytes at the level of the SD after full differentiation has occurred.[27] Nephrin belongs to the immunoglobulin superfamily, having a single putative transmembrane domain, a short intracellular N-terminus, and long extracellular C-terminus.[22] The extracellular C-terminus is predicted to bridge the intercellular space between the interdigitating foot processes, making nephrin a key component of the SD. Nephrin strands contribute to the porous structure of the SD, forming pores of approximately 40 nm.[28] These pores are currently believed to be partly

responsible for the size selectivity of the SD and the glomerular filtration barrier.

Apart from its role as a structural protein, nephrin also appears to participate in intracellular signaling pathways maintaining the functional integrity of the podocyte.[29-31] The SD constitutes a highly dynamic protein complex that recruits signal transduction components and initiates signaling to regulate complex biologic programs in the podocyte. A number of proteins within this signaling platform were identified to interact with nephrin, among them podocin, CD2AP, and TRPC6, all of which are also associated with the development of NS when altered by gene mutations (see later). It is suggested that the plasma membrane of the filtration slit has a special lipid composition comparable to lipid rafts.[32] Lipid rafts are specialized microdomains of the plasma membrane and have a unique lipid content and a concentrated assembly of signal transduction molecules.[33] It was shown that nephrin is a lipid raft–associated protein at the SD and that podocin serves to recruit nephrin into these microdomains. Disease-causing podocin mutations fail to target nephrin into rafts, thus altering nephrin-induced signal transduction.[31] In summary, these studies confirm the extraordinary role of SD proteins in maintaining the glomerular filtration barrier.

Mutations in *NPHS1* were first identified in the Finnish population, leading to the classification of "Finnish type" CNS. Two truncating mutations were found with high frequency in affected Finnish children, suggesting an underlying founder effect in the Finnish population; they are called L41fsX90 (Fin major, truncating the majority of the protein) and R1109X (Fin minor, truncating only a short C-terminal part). In subsequent studies, *NPHS1* mutations were also identified in non-Finnish patients throughout the world, and to date more than 50 different mutations have been described. The Fin major and Fin minor mutations are only rarely observed in non-Finnish patients. Several mutational hot spots were identified affecting the immunoglobulin domains of the nephrin protein.[34] The immunoglobulin domains 2, 4, and 7 appear particularly important for gene function. In addition to the high prevalence in Finland, *NPHS1* mutations are also common among Mennonites in Pennsylvania; 8% of this population are carriers of a heterozygous mutant allele.[35]

Recent studies suggest that CNF may be a genetically heterogenous disorder: in a number of affected patients, *NPHS1* mutations were absent but in some individuals mutations were identified in *NPHS2* (see later). These patients showed the typical histologic features of CNF. However, these results are preliminary and additional studies will have to be performed that include a larger number of patients to confirm a role of *NPHS2* in CNF.

Interestingly, in rare cases a triallelic digenic modus of inheritance was observed in patients with CNS/SRNS: mutations in both *NPHS1* and *NPHS2* were identified with a total of three affected alleles (two *NPHS1* mutations and one *NPHS2* mutation or vice versa).[34] It is speculated that the additional single mutation of the second gene plays a role as a genetic modifier, possibly aggravating the clinical phenotype. These data provide evidence of a functional interrelationship between nephrin and podocin and underscore the critical role that these genes play in regulating glomerular protein filtration.

NPHS2 Gene Mutations Associated with Autosomal Recessive SRNS

The *NPHS2* gene was mapped by linkage analysis in eight families with autosomal recessive SRNS to chromosome 1q25-q31[36] and recessive mutations in *NPHS2* were identified subsequently.[37] NS in these families was characterized by steroid resistance, age at onset between 3 and 5 years, and no recurrence of proteinuria after renal transplantation. *NPHS2* mutations have never been reported in patients with SSNS. Renal histology typically shows FSGS; however, some patients present with only minimal change lesions. In some cases progression from minimal change lesions to FSGS has been demonstrated in repeat biopsies.

NPHS2 encodes for podocin, a 42 kD integral membrane protein expressed in both fetal and mature glomeruli.[37] By electron microscopy and immunogold labeling it was demonstrated that the site of expression is the SD of the podocytes. Because both protein termini are located in the cytosol and podocin is predicted to have only one membrane domain, a hairpinlike structure of the protein was proposed. Interacting with both nephrin and CD2AP, podocin appears to link nephrin to the podocyte cytoskeleton. In patients affected by recessive mutations in *NPHS2*, SD formation is impaired and the typical foot process effacement is visible. These observations suggest that podocin has an important function in maintaining the glomerular filtration barrier. The knockout of *Nphs2* in mice is associated with a phenotype highly reminiscent of the human disease, with podocyte foot process effacement, nephrotic range proteinuria, and chronic renal insufficiency.[38] Podocin, like nephrin, is localized in lipid rafts[32] and is important for recruiting nephrin to these microdomains of the plasma membrane.[31] Some mutations in *NPHS2* impair the ability of podocin to target nephrin to the rafts, especially the most frequent mutation, R138Q, identified in European patients.[31]

Up to now, more than 30 pathogenic mutations have been described in *NPHS2;* most mutations affect the stomatin domain located in the C-terminal part of the protein.[39,40] Mutations in *NPHS2* were first identified in infants with SRNS and rapid progression to ESRD.[37] Subsequently, however, it became evident that defects in podocin can be responsible for SRNS first manifesting at any age from birth to adulthood.[41-43] A partial genotype-phenotype correlation is apparent: whereas the R138Q mutation is typically associated with early-onset NS, other missense mutations (e.g., V180M, R238S) are predominantly found in patients with a later onset of SRNS.[40]

A frequent single nucleotide polymorphism (PM) in the *NPHS2* gene (R229Q) is prevalent in the heterozygous state in approximately 3% of the normal population (range 0.5% to 7%, depending on the genetic background).[44] A common flanking haplotype is associated with the R229Q PM in individuals of widespread ethnic origin, suggesting that the sequence variation arose in a common ancestor long ago.[45] R229Q is present in compound heterozygosity with a single pathogenic *NPHS2* mutation in some patients with FSGS/SRNS. NS manifests late in these individuals, suggesting a reduced pathogenicity when compared with true/nonpolymorphic *NPHS2* mutations. R229Q is therefore considered a nonneutral PM enhancing the susceptibility to FSGS in

association with a second mutant *NPHS2* allele.[45] Moreover, in a large study of more than 1500 individuals of the general population, the R229Q PM was significantly associated with the prevalence of microalbuminuria, a risk factor for developing chronic renal insufficiency and cardiovascular events.[46] In vitro studies have demonstrated that R229Q podocin shows decreased binding to its interacting protein partner, nephrin.[45] These interesting findings underline the functional importance of podocin for the glomerular filtration barrier, with even subtle changes of the amino acid sequence impairing its proper function.

The role of R229Q in the homozygous state is discussed controversially, however. Some authors identified R229Q in the homozygous state in SRNS patients with no other pathogenic mutations in *NPHS2*. In several studies involving very large control groups, no healthy control individual displayed with R229Q in the homozygous state. Only one homozygous control individual was identified out of 1577 samples in the study of Pereira et al.[46] These observations strongly suggest that R229Q homzygosity is disease causing.[40] However, given an average allele frequency of 3%, the frequency of homozygous carriers should amount to 0.1% in the general population, whereas SRNS is much less frequent in demographic terms. Hence R229Q homozygosity alone cannot be sufficient to cause disease, and other genetic or nongenetic factors most likely play a modifying role in disease manifestation in homozygous carriers.

LAMB2 Gene Mutations Associated with Pierson Syndrome

Pierson syndrome is characterized by CNS caused by DMS and peculiar eye abnormalities, including a typical nonreactive narrowing of the pupils (microcoria) (Figure 13-3), but also additional lens and corneal abnormalities.[47] Recently, recessive mutations in *LAMB2* on chromosome 3p21 were identified as the underlying genetic defect.[48] *LAMB2* encodes for the protein laminin β2, one component of the trimeric laminins in the kidney that crosslink the basolateral membrane of the podocyte to the GBM. Most disease-associated alleles identified in Pierson patients were truncating mutations leading to loss of laminin β2 expression in the kidney.[48] Ocular laminin β2 expression in unaffected controls was strongest in the intraocular muscles, corresponding well to the characteristic hypoplasia of ciliary and pupillary muscles observed in affected patients. Subsequent genotype-phenotype studies revealed that some mutations in *LAMB2*, especially hypomorphic missense mutations, can be associated with a phenotypic spectrum that is much broader than previously anticipated, including isolated CNS or CNS with minor ocular changes different from those observed in Pierson syndrome.[49] Fetal ultrasound in four consecutive fetuses of a family with Pierson syndrome and positive *LAMB2* mutation analysis consistently revealed marked hyperechogenicity of the kidneys and variable degrees of pyelectasis by 15 weeks of gestation.[50] Placentas were significantly enlarged. Hydrops fetalis due to severe hypalbuminemia demonstrated by chordocentesis occurred in one fetus, and anencephaly was detected in another. Development of oligohydramnios indicated a prenatal decline of renal excretory function. From these studies it can be concluded that mutational analysis in *LAMB2* should also be considered in isolated CNS if no mutations are found in *NPHS1*, *NPHS2*, or *WT1*, and in cases with prenatal onset of nephrotic disease with typical sonomorphologic findings of the kidneys and the development of oligohydramnios.

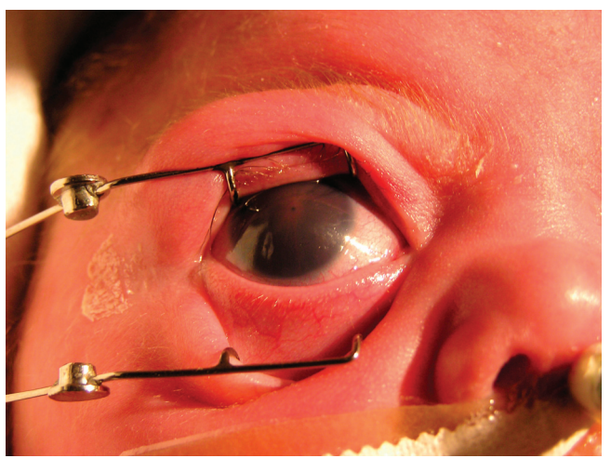

Figure 13-3 Typical aspect of a patient with Pierson syndrome and microcoria. (Courtesy Kveta Blahova, Pediatric Clinic, Charles University, Prague, Czech Republic.)

LMX1B Gene Mutations Associated with Autosomal Dominant Nail-Patella Syndrome

Nail-patella syndrome (NPS) or onychoosteodysplasia is caused by dominant mutations in the *LMX1B* gene, located on chromosome 9q34.1 and encoding the LIM-homeodomain protein Lmx1b. Lmx1b plays a central role in dorsal/ventral patterning of the vertebrate limb, and targeted disruption of Lmx1b results in skeletal defects including hypoplastic nails, absent patellae, and a unique form of renal dysplasia.[51] Prominent features of affected children are dysplasia of nails and absent or hypodysplastic patellae. In many patients, iliac horns, dysplasia of the elbows, glaucoma, and/or hearing impairment are also detected. *LMX1B* is highly expressed in podocytes, and patients can also display an involvement of the kidney comprising proteinuria, nephrotic syndrome, or renal insufficiency. Overall, nephropathy is reported in approximately 40% of affected patients (microalbuminuria or overt proteinuria)[52] but ESRD in less than 10%.[53] Interestingly, renal involvement appears significantly more frequent in females and in patients with a positive family history of NPS nephropathy.[52] In NPS patients with renal involvement, electron microscopy shows collagen fibrillike deposition in the GBM with typical lucent areas.[54] These characteristic ultrastructural changes can be present even in patients without apparent nephropathy.[55] Large genotype-phenotype studies have demonstrated that individuals with an *LMX1B* mutation located in the homeodomain show a significantly higher frequency of renal protein loss and higher values of proteinuria than subjects with mutations in the LIM domains.[52] However, no clear genotype-phenotype association is apparent for extrarenal manifestations.

Insight into Lmx1b function has been further obtained by the generation of *Lmx1b* knock-out animals.[56] In *Lmx1b*(–/–) mice the expression of GBM collagens is reduced and podocytes have a reduced number of foot processes, are dysplastic, and lack typical SD structures. Interestingly, mRNA and protein levels for CD2AP and podocin are greatly reduced in these kidneys and several *LMX1B* binding sites were identified in the putative regulatory regions of both *CD2AP* and *NPHS2* (encoding podocin).[56] These observations support a cooperative role for Lmx1b, CD2AP, and podocin in foot process and SD formation (see Figure 13-2).

PLCE1 Gene Mutations Associated with Autosomal Recessive Nephrotic Syndrome

A new gene locus for nephrotic syndrome *(NPHS3)* was recently mapped to chromosome 10q23-q24. Positional candidate genes were selected based on increased gene expression levels in a rat glomerulus differential expression database, and six different homozygous truncating mutations in six different kindreds were identified in the *PLCE1* gene.[57] Interestingly, two of the individuals with truncating *PLCE1* mutations entered remission following steroid or cyclosporin A treatment. The observation of a possible responsiveness to immunosuppression in *PLCE1* mutation carriers awaits confirmation in a larger number of affected patients.

PLCE1 codes for the enzyme phospholipase C epsilon-1, which is involved in intracellular signal transduction. *PLCE1* is widely expressed in many tissues, including the podocytes. The knock-down of *pcle1* in zebrafish is associated with the development of podocyte foot process effacement and edematous outer appearance of the fish,[57] confirming a specific role of phospholipase C epsilon-1 in the maintenance of the glomerular filtration barrier. Still, the pathogenesis of isolated podocyte damage and development of proteinuria in patients lacking phospholipase C epsilon-1 remains to be elucidated.

Recessive SRNS with Sensorineural Deafness

In 2003 Ruf et al. mapped a novel gene locus for recessive SRNS on chromosome 14q24.2 in a large consanguineous Palestinian kindred with SRNS and deafness.[58] The causative genetic defect is still unidentified.

HEREDITARY DISORDERS WITH LATE-ONSET NEPHROTIC SYNDROME

Hereditary late-onset FSGS is a heterogeneous condition generally transmitted in an autosomal dominant fashion. Three disease loci (FSGS1, FSGS2, and FSGS3) have been mapped in affected families, and as a result the responsible genetic defects have been identified.

ACTN4 Gene Mutations

In 1998 a locus for autosomal dominant late-onset FSGS (FSGS1) was mapped to chromosome 19q13 (FSGS1),[59] and mutations in *ACTN4* were identified as the underlying pathogenic cause.[60] *ACTN4* encodes for α-actinin-4, an actin-bundling protein of the cytoskeleton highly expressed in podocytes. Both a knock-down and an overexpression transgenic mouse model have been established for *Actn4*, demonstrating proteinuria and podocyte alterations. It was therefore discussed that α-actinin-4 plays an important role for the cytoskeletal function of the podocyte. Young knock-out mice present with focal areas of foot process effacement, and older animals present with diffuse effacement and globally disrupted podocyte morphology.[61] Moreover, *Actn4* was shown to be upregulated in the kidneys of different animal models of proteinuria. Human *ACTN4* mutations were identified in three different families with FSGS.[60] The clinical course in affected family members was characterized by progressively increasing proteinuria starting in adolescence and developing into FSGS and chronic renal insufficiency later in adult life. ESRD was observed in a number of affected individuals. All *ACTN4* mutations identified so far represent nonconservative amino acid substitutions affecting the actin-binding domain of α-actinin-4. In vitro studies demonstrated that mutant α-actinin-4 binds filamentous actin more strongly than wild-type protein. Based on this observation it was proposed that dominant mutations in *ACTN4* interfere with the maintenance of podocyte architecture: a proper organization of the cytoskeleton seems to be important for normal functioning of podocyte foot processes. Interestingly, however, not all mutation carriers of the families reported by Kaplan et al. displayed with a renal phenotype. The observed incomplete penetrance suggests that additional factors (genetic or nongenetic) are involved in the pathogenesis, which in conjunction with a mutation in *ACTN4* lead to the manifestation of FSGS. *ACTN4* mutations may confer disease susceptibility, as also discussed for mutations in *CD2AP* and *TRPC6*. However, mutations in *ACTN4* represent a rare cause of hereditary FSGS, accounting for approximately 4% of familial FSGS.[62]

TRPC6 Gene Mutations

In 1999 a second gene locus for autosomal dominant FSGS (FSGS2) was mapped to chromosome 11q21-q22 using a 399-member Caucasian kindred of British heritage dating back seven generations.[63] Fourteen deceased family members had suffered from ESRD, 14 living family members were on dialysis or had undergone renal transplantation, and 3 individuals were proteinuric. Six years later, the responsible gene, *TRPC6,* was identified.[64,65] *TRPC6* encodes the transient receptor potential cation channel TRPC6 that is thought to mediate capacitative calcium entry into cells. Expression analysis revealed that TRPC6 is highly expressed in the kidney and also in podocytes at the site of the SD. A dominant missense mutation was identified in the original family studied by Winn et al., and five additional families with mutations in TRPC6 were characterized by Reiser et al. Two of the missense mutations in the latter study were shown to increase the current amplitudes of TRPC6, consistent with a gain-of-function effect of the mutations. Interestingly, however, both studies describe carrier individuals with a normal renal phenotype, pointing to an incomplete penetrance of the mutations. *TRPC6* mutations have been identified in very few children; early disease onset seems to be exceptional. So far it is unknown how the dysfunction of a cation channel is related to the development of podocyte damage and loss of the glomerular filtration barrier. One hypothesis is related to the observation that MEC-2, a C. *elegans* homologue of podocin, participates in the mechano-

sensation of the worm. MEC-2 is physically and functionally linked to ion channels, transducing the signals of mechanosensation. Because TRPC6 interacts with podocin and nephrin at the SD, it was proposed that podocin takes part in mechanosensation processes at the glomerular filtration barrier, transducing signals to TRPC6 that in turn modulates intracellular calcium concentrations in the podocyte. Nephrin, on the other hand, is thought to stimulate different pathways of the intracellular signaling machinery. Therefore, a complex protein network involving nephrin, podocin, CD2AP, and the cation channel TRPC6 is established to maintain the SD structure of the foot process. Mutations in TRPC6 likely affect this functional network by altering the intracellular calcium concentration of the podocyte.

CD2AP Gene Mutations

In 1999, FSGS3 was shown to map to chromosome 6 and reported to be caused by haploinsufficiency for *CD2AP*.[66] *CD2AP* encodes for the CD2-associated protein CD2AP, an actin-binding protein that was originally identified as a cytoplasmic ligand of the CD2 receptor on T and natural killer cells. *CD2AP* knock-out mice presented not only with impaired immune functions but also with severe NS and FSGS, accompanied by mesangial hypercellularity and extracellular matrix deposition.[67] Electron microscopy showed the typical loss of podocyte foot process integrity with process effacement and loss of the SD structure. Screening in FSGS patients led to identification of a dominant *CD2AP* mutation (a 2-bp substitution altering the exon 7 splice acceptor site) in two adult patients with late-onset FSGS.[66] An enhanced disease susceptibility for FSGS conferred by the change in CD2AP expression was postulated as the underlying pathogenic mechanism. CD2AP interacts with nephrin and both proteins localize to lipid rafts in the plasma membrane,[32] suggesting that CD2AP is required to connect nephrin (and thus the SD) to the cytoskeleton of the podocyte. An impairment of CD2AP function might be associated with enhanced cytoskeletal fragility, predisposing to podocyte damage. Since the initial description of *CD2AP* mutations in two patients, no additional human mutations have been reported. Hence the overall role of CD2AP for human disease remains to be elucidated.

SYNDROMAL DISORDERS ASSOCIATED WITH NEPHROTIC SYNDROME

A large number of syndromes have been described on clinical grounds with patients displaying proteinuria (steroid resistant) in addition to various extrarenal manifestations. Renal histology usually reveals FSGS lesions, but DMS may also be observed. Classification of the syndromal entity is usually based on characteristic accompanying extrarenal manifestations. A genetic basis has been identified in only a minority of these syndromes. Here we discuss two important syndromes that invariably present with SRNS: Schimke syndrome and Galloway-Mowat syndrome.

Schimke Syndrome

Schimke immunoosseous dysplasia maps to chromosome 2q34-36 and is caused by recessive mutations in the *SMARCAL1* gene.[68] *SMARCAL1* encodes the SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily a-like protein 1, a protein involved in the remodeling of chromatin to change nucleosome compaction for gene regulation, replication, recombination, and DNA repair. The clinical phenotype of Schimke immunoosseous dysplasia is characterized by growth retardation caused by spondyloepiphyseal dysplasia, a slowly progressive immune defect, cerebral infarcts, skin pigmentation, and SRNS beginning in childhood. FSGS lesions are frequently observed in kidney biopsy specimens and the majority of patients progress to ESRD. However, disease severity and age at onset follow a continuum from early onset and severe symptoms, with death early in life, to later onset and mild symptoms, with survival into adulthood. Genotype-phenotype studies suggest that recessive loss-of-function mutations (frameshift, stop, and splice-site mutations) are generally associated with a more severe course of the disease, whereas some missense mutations allow retention of partial *SMARCAL1* function and thus cause milder disease.[68]

Galloway-Mowat Syndrome

Galloway-Mowat syndrome (GMS) is characterized by microcephaly and other brain anomalies, severe mental retardation, and early-onset NS (CNS).[69] Both FSGS and DMS were observed in kidney biopsies of affected individuals.[69,70] An important number of patients also show hiatus hernia. Both males and females are affected and an occurrence in siblings of the same family has been reported. These observations point to a possible autosomal recessive mode of inheritance, but no gene locus has thus far been identified. However, as different genetic research groups work hard to map the causative gene locus, new insights into the pathology of GMS can be anticipated.

CLINICAL ASPECTS

Clinical aspects of NS are discussed in detail in earlier chapters. Here we want to focus on issues specific for genetic forms of SRNS.

Therapeutic Implications

In general, therapy for SRNS is demanding. Numerous immunosuppressive agents have shown some efficacy in a fraction of the SRNS population; these include cyclophosphamide, azathioprine, cyclosporin, and mycophenolate mofetil, mostly in combination with glucocorticoids. However, genetically determined forms of SRNS have proven insensitive to immunosuppressive interventions, which is pathophysiologically explained by the presence of intrinsic defects in podocyte architecture and function. Hence it is suggested that children with hereditary SRNS, especially with *NPHS2* mutations, be spared from any form of immunosuppressive treatment. Conversely, *NPHS2* mutations have never been reported in patients with SSNS, so screening for this mutation seems not to be indicated even in patients with reduced steroid sensitivity (such as frequent relapsers or those who are steroid dependent).

Antiproteinuric pharmacological treatment with ACE inhibitors or AT1 receptor blockers is probably effective in

slowing down the progression of renal insufficiency, although efficacy has not been formally proven in carriers of individual mutations.

Living-Related Donor Transplantation

Living-related kidney transplantation is generally considered the therapy of choice in pediatric patients with ESRD. However, in patients affected by SRNS caused by germline mutations in podocyte genes, several aspects need to be considered. First, it is yet unknown how kidneys of a heterozygous donor behave and develop in a recipient with recessive SRNS. Parents of affected children with recessive SRNS each carry one mutant allele that is also present in the transplanted kidney. It could be speculated that these kidneys are more easily prone to develop proteinuria if other proproteinuric factors (e.g., arterial hypertension, salt-rich diet) are superposed. So far animal models of SRNS do not support this hypothesis and comprehensive human data addressing this question are lacking. Consequences for the donor should also be considered. It is still unknown whether the prognosis of the remaining single kidney in the heterozygous parental donor is impaired by the gene mutation. Again, the remaining heterozygous kidney may be more susceptible to proteinuric disease than are single kidneys of individuals without mutations. Up to now our experience with living-related donor transplantation in hereditary SRNS is limited and does not support a restriction in affected children. Still, careful surveillance of both donor and recipient seems advisable.

In families of patients affected by autosomal dominant late-onset SRNS, only one of the parents is a carrier of the pathogenic sequence variation. Genetic testing of family members will help delineate mutation carriers in the family. If the mutation occurred as a de novo mutation in the patient, both parents are equally suitable for living-donor transplantation from a genetic point of view.

Recurrence of Nephrotic Syndrome after Renal Transplantation

Many investigators have studied the pathogenesis of increased glomerular permeability and recurrence of proteinuria after transplantation in FSGS. In general, recurrence of proteinuria after renal transplantation is observed in approximately 30% of FSGS patients.[71] This risk appears higher in children than in adult patients.[72] Affected patients display with proteinuria, which is often in the nephrotic range. Frequently, proteinuria recurs within a few days after renal transplantation. In children, the mean time to recurrent proteinuria is 14 days posttransplant.[73] Recurrence of proteinuria/FSGS following renal transplantation negatively impacts graft survival in both children and adult patients. Risk factors are an age less than 15 years, rapid progression of renal insufficiency, and diffuse mesangial proliferation in the initial biopsy of the native kidney.[74] In nonhereditary FSGS/SRNS, recurrence of proteinuria has been discussed to follow a T-cell dysfunction and production of proteinuric circulating factor(s), such as using an in vitro bioassay of glomerular permeability to albumin. Savin et al. proposed the existence of a circulating proteinuric factor that predisposes to the development of posttransplant proteinuria recurrence.[75]

In *NPHS2*-associated SRNS/FSGS, recurrence of posttransplant proteinuria is a rare phenomenon, observed in less than 10% of transplant patients.[39,40] Interestingly, the in vitro test of glomerular permeability has also shown high values in a few patients with proven recessive *NPHS2* mutations and posttransplant recurrence,[76] and an important role of circulating serum factors has been discussed to explain this phenomenon in hereditary SRNS. However, these factors were not identified in all patients with *NPHS2* mutations and with recurrence of proteinuria after transplantation.[77] Therefore, possible proteinuric factors are not the only cause of posttransplant recurrence in patients with *NPHS2* mutations.[78] The biochemical nature of proteinuric factors has yet to be determined. Identification of a homozygous truncating *NPHS2* mutation in one patient with posttransplant NS prompted the search for antipodocin antibodies, but all results were negative, excluding a de novo glomerulonephritis as the underlying cause.[40] Antipodocin antibodies were also not identifiable in patients with *NPHS2* missense mutations and posttransplant NS.

In CNF, the risk of a recurrence of proteinuria after transplantation seems to be important: it was demonstrated that especially patients affected by the Fin major mutation have a risk of approximately 25% of posttransplant NS. Subsequent studies revealed that the pathogenesis of this recurrence is related to the development of antinephrin antibodies directed against the wild-type nephrin protein residing in the transplanted kidney,[79] analogous to the anti-GBM antibodies against type IV collagen causing posttransplant de novo glomerulonephritis in patients with Alport syndrome. Treatment options of posttransplant NS in these patients are scarce; a subset of patients seems to respond to cyclophosphamide.[79]

Genetic Counseling

Positive results of mutational analysis in pediatric patients with SRNS should be followed by adequate genetic counseling. This demands close collaboration between pediatric nephrologists and human geneticists. Parents of children affected by recessive disease will have a 25% chance of giving birth to another affected child. In parents of children with dominant disease, this risk amounts to 50% (with the exception of patients with de novo mutations; the risk of recurrence in these families is very low). Parents of affected children need to be informed that treatment options are limited in hereditary SRNS and that renal function may deteriorate rapidly. Close monitoring of renal function and early treatment of complications of chronic renal insufficiency are advised. In autosomal dominant FSGS, genetic counseling might be difficult because of incomplete penetrance and variable expressivity. It seems that individual mutation carriers can be affected to differing degrees, with an obvious mild phenotype in some family members and ESRD in others. Genetic counseling is not only important for parents but also for the affected child. Children with recessive disease will transmit a heterozygous mutation to their own future children, but as long as the other parent is not a mutation carrier, all offspring will be healthy. Patients affected by dominant FSGS will transmit the pathogenic mutation in 50% of cases, but offspring carrying the mutation might also be affected by FSGS.

In some cases established genotype-phenotype correlations might be helpful to estimate the risk of a more severe clinical course. In *NPHS2*-associated SRNS, for example, some mutations have been associated with early onset and aggravated clinical course, whereas other mutations were shown to be less pathogenic.[40] For other disease entities, the analysis of clinical symptoms of other affected family members can help predict the severity of the disease: in NPS, the risk of having a child with NPS nephropathy is about 1 : 4 and the risk of having a child in whom renal failure will develop is about 1 : 10 if NPS nephropathy occurs in other family members.[53] Genetic counseling is especially important in families affected by NS with serious prognosis. In children affected by CNS with female outer appearance, mutation analysis in *WT1* is mandatory in order to rule out risk of Wilms' tumor development.

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