REVIEW ARTICLE

MECHANISMS OF DISEASE

Hereditary Proteinuria Syndromes and Mechanisms of Proteinuria

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THE INHERITED FORMS OF PROTEINURIA COMPRISE A HETEROGENEOUS group of rare renal diseases in which glomerular dysfunction and proteinuria are prominent. Despite the rarity of hereditary proteinuria syndromes, genetic, biochemical, and structural studies of these diseases have made important contributions to our knowledge of how the normal glomerular filter works and the mechanisms of proteinuria.

The courses of these diseases can vary. Some patients present with severe proteinuria and congenital nephrotic syndrome, whereas others have only moderate proteinuria and focal segmental glomerulosclerosis. Regardless of its cause, the disease often progresses to end-stage renal disease. Classification of these syndromes has been difficult because the age at onset and the clinical manifestations vary, but in recent years, considerable progress has been made in determining the genetic causes of these conditions. There can be overlap between the diseases: mutations in the same gene can lead to either congenital nephrotic syndrome or focal segmental glomerulosclerosis. Therefore, we refer to these diseases as hereditary proteinuria syndromes. From a clinical standpoint, it is important to know that some hereditary proteinuria syndromes respond to therapy, whereas others do not. For this reason, genetic testing, which is available for some hereditary proteinuria syndromes, should be performed whenever possible. Knowledge of the mechanisms of glomerular filtration and proteinuria is still limited, but this field is the subject of intensive and productive research. This review summarizes recent progress in studies of the glomerular filter and the causes of hereditary proteinuria syndromes.

THE GLOMERULAR FILTRATION BARRIER

The primary causes of hereditary proteinuria syndromes are insults to the filtration barrier in the glomeruli of the kidney cortex (Fig. 1A and 1B). This barrier has three layers: the fenestrated endothelium, the glomerular basement membrane, and the podocytes, together with a slit diaphragm between the interdigitating foot processes of the podocytes (Fig. 1C and 1D). The filtration barrier is believed to be a size-selective and charge-selective filter,¹⁻⁴ but the molecular basis of its function remains unknown.

FENESTRATED ENDOTHELIUM

The function of the fenestrated endothelium in filtration is poorly understood. The endothelial cells have numerous openings, 70 to 100 nm in diameter, termed "fenes-trae," which in mature glomeruli do not have a visible diaphragm that would constitute a physical barrier for macromolecules in the plasma. Recent studies in

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Figure 1 (facing page). Glomerular Filtration System. Each kidney contains about 1 million glomeruli in the renal cortex (Panel A). Panel B shows an afferent arteriole entering Bowman's capsule and branching into several capillaries that form the glomerular tuft; the walls of the capillaries constitute the actual filter. The plasma filtrate (primary urine) is directed to the proximal tubule, whereas the unfiltered blood returns to the circulation through the efferent arteriole. The filtration barrier of the capillary wall contains an innermost fenestrated endothelium, the glomerular basement membrane, and a layer of interdigitating podocyte foot processes (Panel C). In Panel D, a cross section through the glomerular capillary depicts the fenestrated endothelial layer and the glomerular basement membrane with overlying podocyte foot processes. An ultrathin slit diaphragm spans the filtration slit between the foot processes, slightly above the basement membrane. In order to show the slit diaphragm, the foot processes are drawn smaller than actual scale.

genetically modified mice suggest that podocytederived vascular endothelial growth factor has a major role in the development of the endothelium and the maintenance of its fenestrations.⁵ Glomerular endothelial cells have a glycocalyx on their surface containing negatively charged sialoproteins and proteoglycans,^{6,7} but there is no direct evidence that the glycocalyx has a role in filtration.

GLOMERULAR BASEMENT MEMBRANE

The glomerular basement membrane is an acellular matrix with a thickness of 300 to 350 nm that provides structural support for the capillary wall. Its main components are type IV collagen, proteoglycans, laminin, and nidogen.8,9 In the fetus, the triple-helical type IV collagen molecules of the glomerular basement membrane contain α 1(IV) and α 2(IV) chains in a 2:1 ratio, but this form of collagen is later replaced by adult-type molecules containing α 3(IV), α 4(IV), and α 5(IV) chains in a 1:1:1 ratio.9 The highly cross-linked type IV collagen network provides tensile strength to the membrane but probably does not contribute to the size-selectivity or charge-selectivity of the glomerular filter. This view is supported by the finding that mutations in adult type IV collagen lead to distortion of the structure of the glomerular basement membrane in patients with Alport's syndrome, which includes hematuria as a renal manifestation, but usually cause only mild proteinuria.9,10

Electron-microscopical studies involving a tracer have identified anionic sites in the glo-

merular basement membrane.^{2,11} These sites are believed to be located on the heparan sulfate and chondroitin sulfate side chains of perlecan and agrin.12,13 The anionic charges have been thought to be important for filtration, since enzymatic removal or reduction in the number of the charges results in proteinuria.14,15 However, charges in the glomerular basement membrane itself may not have a crucial role, because intravenous glycosaminoglycan-degrading enzymes can affect glycosaminoglycans in all three layers of the filtration barrier. Moreover, genetically engineered mice whose glomerular basement membrane contains heparan sulfate-deficient perlecan or lacks agrin do not have proteinuria.^{16,17} These animals, however, are prone to proteinuria when challenged with an albumin overload.18

Laminins are large, heterotrimeric proteins that are important for cellular differentiation and adhesion. They also have a structural function: they assemble themselves into a laminin network in many types of basement membrane. In the fetal glomerular basement membrane, an isoform of laminin, laminin-10 (α 5: β 1: γ 1), is replaced after birth by laminin-11 (α 5: β 2: γ 1).¹⁹ Ablation of the laminin β^2 gene in mice causes a lack of laminin-11, proteinuria, and neonatal death.²⁰ Recently, mutations of the laminin β 2 gene were shown to cause Pierson's syndrome, an early, lethal form of congenital nephrotic syndrome.²¹ Laminin-11 is therefore indispensable for the function of the glomerular basement membrane.

How the glomerular basement membrane contributes to macromolecular filtration is not clearly understood. Current data do not suggest an important role for type IV collagen or glomerular basement membrane proteoglycans in this process, but the laminin-11 isoform in adult glomerular basement membranes is somehow important for filtration.

THE PODOCYTE SLIT DIAPHRAGM

The podocyte slit diaphragm has an important and direct role in glomerular filtration. Some of its protein components are involved in the mechanism of proteinuria. These proteins form a complex that contributes to the structure of the slit diaphragm, connects the diaphragm to the intracellular actin cytoskeleton, and participates in signaling related to turnover of the glomerular filter. Most of these proteins are essential for a

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Figure 2. Components of the Slit-Diaphragm Protein Complex in Podocyte Foot Processes.

As shown in Panel A, nephrin has a short intracellular domain, a transmembrane domain (TM), and an N-terminal extracellular domain with a proximal fibronectin type III-like motif (FN) and eight IgG-like motifs numbered from the N-terminal. Panel B shows homophilic interactions among nephrin molecules. Extracellularly, molecules from adjacent foot processes are believed to interact in the center of the slit to form the zipper-like backbone of the slit diaphragm. This type of assembly could allow pores to be located on both sides of the central density. As shown in Panel C, the transmembrane Neph1 and Neph2 molecules each contain five extracellular IgG-like motifs. As shown in Panel D, the Neph molecules are believed to have homophilic interactions with identical Neph molecules and heterophilic interactions with adjacent nephrin molecules. However, Neph1 and Neph2 do not interact with each other. As shown in Panel E, FAT1 and FAT2 are transmembrane proteins of more than 500 kD that contain 34 consecutive extracellular cadherin-like motifs. Their modes of interaction with other slit-membrane proteins have not been characterized. As shown in Panel F, podocin is an integral membrane protein of about 30 kD, with its N- and C-terminals located intracellularly.

functional slit diaphragm and glomerular filtration, since mutation or inactivation of the corresponding genes causes proteinuria.

Nephrin

Nephrin was the first slit-diaphragm protein to be identified, and the nephrin gene is mutated in congenital nephrotic syndrome of the Finnish type (CNF, or nephrotic syndrome type 1 [NPHS1]).²² In the kidney, only podocytes express nephrin, 23-25 and inactivation of the nephrin gene in the mouse causes massive proteinuria, absence of a slit diaphragm, and neonatal death.²⁶ Nephrin has a short intracellular domain, a transmembrane domain, and an extracellular domain with eight distal IgG-like motifs and one proximal fibronectin type III-like motif (Fig. 2A). Nephrin molecules interact with one another in a homophilic fashion.27 The length of the extracellular domain of nephrin is about 35 nm, and nephrin molecules from adjacent foot processes are thought to interact in the middle of the slit to form a filtering structure (Fig. 2B).²⁸ Intracellularly, phosphorylation of tyrosine in the cytoplasmic tail of nephrin by Src kinase (Src is a tyrosine kinase with a critical role in cell signaling) initiates a signaling cascade and seems to promote antiapoptotic signals.^{29,30} The importance of Fyn-dependent phos-

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phorylation of nephrin (Fyn is a member of the Src family of protein tyrosine kinases) is underlined by the fact that proteinuria and podocyte effacement develop in mice lacking Fyn kinase.³¹

Neph1 and Neph2

Neph1 and Neph2 are structurally related to nephrin; each has five extracellular IgG-like motifs (Fig. 2C). They belong to a family of transmembrane proteins (Neph1, Neph2, and Neph3, also termed filtrin) that are found in many tissues.³²⁻³⁴ Neph1 and Neph2 are located in the slit diaphragm,^{35,36} and in vitro data suggest that nephrin can form heterodimers with Neph1 or Neph2 through their extracellular domains, but that Neph1 and Neph2 do not interact with each other (Fig. 2D).³⁷ When phosphorylated, these proteins participate in intracellular signaling.^{38,39} Mice deficient in Neph1 have proteinuria and die within the first eight weeks of life,³² but the functional significance of Neph2 or Neph3 is unknown.

FAT1 and FAT2

FAT1 and FAT2 are large, slit-diaphragm transmembrane proteins containing 34 tandem cadherin-like repeats (Fig. 2E).^{40,41} The absence of FAT1 in mice causes loss of slit diaphragms, and proteinuria; forebrain and ocular defects; and perinatal death.⁴² Lack of FAT2 causes only proteinuria.⁴¹ P-cadherin and junctional adhesion molecule 4 have also been identified in the slit diaphragm,^{43,44} but the former is not indispensable for glomerular filtration,⁴⁵ and the role of the latter remains to be elucidated.

Podocin

Positional cloning of the gene mutated in corticosteroid-resistant congenital nephrotic syndrome (*NPHS2*) led to the discovery of podocin, which is located solely in the slit-diaphragm region.^{46,47} It is a hairpin-shaped integral membrane protein with both ends directed into the intracellular space (Fig. 2F). Podocin interacts with the intracellular domains of nephrin and Neph1 and with CD2-associated protein (CD2AP).^{33,48} Severe proteinuria develops in podocin-knockout mice, and they die within a few days after birth.⁴⁹

CD2AP

CD2AP is an intracellular protein initially characterized as a T-lymphocyte CD2 adapter protein.⁵⁰ However, most CD2AP-knockout mice die of a nephrotic syndrome–like disease at six to seven weeks of age, and the protein is located in the podocyte slit-diaphragm region of the glomerulus.^{51,52} Persons who are heterozygous for a defective *CD2AP* allele have a complex renal phenotype, and polymorphisms in the human gene have been associated with the development of glomerulonephritis and glomerulosclerosis.⁵³ Thus, *CD2AP* can be viewed as a susceptibility gene for glomerulonephritis. CD2AP may interact with the intracellular domains of nephrin and podocin, but the protein has also been associated with endocytosis.^{48,52,53} CD2AP is also involved in slit-diaphragm signaling.³⁰

Other Protein Constituents of the Slit Diaphragm

ZO-1, a widely expressed intracellular protein connected with epithelial tight junctions,⁵⁴ is also located in the slit-diaphragm region and can interact with Neph family proteins in vitro.²⁹ The role of ZO-1 in the slit-diaphragm protein complex is not known. A member of the LAP (leucinerich repeats and PDZ domains) protein family, densin, and MAGI-1 have also been localized to the slit-diaphragm region.^{55,56} The functions of these proteins are unknown. It has also been reported that nephrin forms a complex with cadherins, p120 catenin, and the scaffolding proteins ZO-1, CD2AP, and calcium calmodulin-dependent serine protein kinase (CASK).⁵⁷

The discovery of the specific components of the slit-diaphragm protein complex has led to new insights into the biology of the filtration barrier and the mechanisms of proteinuria. The fact that most of these proteins are crucial for normal development and function emphasizes the importance of the slit diaphragm in determining the molecular-sieving characteristics of the glomerulus.

Structure of the Slit Diaphragm

Does the slit diaphragm (Fig. 3A and 3B) have a true porous filter structure? On the basis of their electron-microscopical findings, Rodewald and Karnovsky⁶⁰ proposed that the slit diaphragm has an ordered, zipper-like structure with pores that are smaller in diameter than the albumin molecule when viewed en face. This model was called into question by the results of deep-etching experiments with unfixed quick-frozen tissue,

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Figure 3. Electron-Microscopical Imaging (Panels A and B) and Electron-Tomographic Imaging (Panel C) of the Podocyte Slit Diaphragm.

In Panel A, a cross section of a human glomerular capillary shows filtration slits with slit diaphragms (arrows) between the podocyte foot processes (FPs). The glomerular basement membrane (GBM), and an endothelial cell (E) are also shown. The scale bar represents 250 nm. (Modified from Lahdenkari et al.⁵⁸ with the permission of the publisher.) Panel B shows slit diaphragms (arrows) at a higher magnification. The scale bar represents 150 nm. (Modified from Lahdenkari et al.⁵⁸ with the permission of the publisher.) Panel C shows a thin, three-dimensional electron tomogram of the mouse slit diaphragm (SD) en face. Cross-strands (arrows) extend from cross-cut podocyte surface membranes (M) to a central density (CD), forming lateral pores (Ps). The tomogram is a surface-rendered reconstruction. For comparison with the diameter of the pores at the same magnification, a space-filled model (yellow) of the crystal structure of a serum albumin molecule has been superimposed. The scale bar represents 10 nm. (Modified from Wartiovaara et al.⁵⁹ with the permission of the publisher.)

which suggested that the slit diaphragm had an even, sheet-like structure.61 However, recent analysis of the slit diaphragm with a novel high-resolution electron-tomographic method⁵⁹ has demonstrated that this thin layer contains convoluted strands that cross the midline of the filtration slit and most often form zipper-like sheets with pores the diameter of the albumin molecule or smaller located on both sides of a central density (Fig. 3C). Immunoelectron microscopy and electron tomography have been used together to show that the distal IgG1 and IgG2 motifs of nephrin are in the central region of the slit diaphragm (Fig. 4A, 4B, and 4C). Moreover, immunolabeled nephrin molecules in solution (Fig. 4D) resemble a class of slit-diaphragm strands detected in situ by the same methods.59

Taken together, the molecular and electrontomographic data suggest that proteins of the slit diaphragm form a zipper-like structure with a constant width of approximately 40 nm (Fig. 5). The exact locations and interactions of Neph1, Neph2, FAT1, and FAT2 among these interacting proteins are unknown. These proteins interact intracellularly with several proteins that connect with the cytoskeleton or participate in cell signaling. It seems plausible that a combination of protein crystallography and high-resolution electron tomography could be used to elucidate the three-dimensional structure of slit-diaphragm molecules.

If, as seems likely, the slit diaphragm is a true size-selective filter, the important question is why it does not clog. We do not have a complete answer to this question, but it is possible that the negative charges of glycosaminoglycans in the glomerular basement membrane and on podocyte cell surfaces, a gel-exclusion effect,⁶² or some other as yet unidentified mechanism acts to repel proteins from the slit diaphragm and thus prevents clogging.

HEREDITARY PROTEINURIA SYNDROMES

Table 1 summarizes the classification of currently known genetically determined hereditary proteinuria syndromes. Some of these syndromes can be diagnosed accurately from their clinical manifestations, but there are overlapping phenotypes. Therefore, it is important to perform a genetic analysis whenever appropriate tests are available.

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end (N-) of the strand formed of globular substructures, such as the solid label in Panel C. The scale bar represents 5 nm. (All panels except Panel D have been modified from Wartiovaara et al.⁵⁹ with the permission of the publisher.)

CNF

CNF (Online Mendelian Inheritance in Man [OMIM] number 256300)63 is caused by mutations in the nephrin gene.^{22,64} The disease is particularly common in Finland, where the incidence is 1 in 8200 births,65 but it occurs worldwide. Affected persons have massive proteinuria even in utero, and the nephrotic syndrome develops soon after birth. Children with CNF are usually born prematurely; the weight of the placenta is almost invariably more than 25 percent of the weight of the child at birth. Typically, hypoalbuminemia, hyperlipidemia, abdominal distention, and edema appear in affected infants soon after birth.⁶³ Electron microscopy and electron tomography (Fig. 6) show effacement of the podocytes, a narrow slit, and absence of the slit diaphragm.^{59,63}

nephrin, which leads to the absence or malfunction of the slit diaphragm and loss of the sizeselective slit filter. About 70 different mutations have been described in affected persons.64,66,67 In the Finnish population, two nonsense founder mutations (Fin-major and Fin-minor) account for more than 94 percent of all mutations. Outside Finland, in a large number of countries, most mutations are single-nucleotide missense mutations, but nonsense and splice-site mutations, as well as deletions and insertions, have also been described.^{64,67} A few missense nephrin mutations have been associated with a phenotype of mild focal segmental glomerulosclerosis rather than a phenotype of congenital nephrotic syndrome, a finding that emphasizes the need for genetic analysis to make the correct diagnosis.

CNF is caused by the absence of functional

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CNF is lethal. Immunosuppressive therapy with corticosteroids and cyclophosphamide does not induce a remission. Therefore, at present, all treatment should be geared toward kidney transplantation, the only curative approach.⁶⁸ Patients with the Fin-major nonsense mutation do not have a response to treatment with angiotensin-converting–enzyme inhibitors or antiinflammatory drugs. However, because other patients with "milder" mutations may have a response to such therapy,

it should be considered for patients with missense mutations.⁶⁹ Successful transplantation is curative, and several patients who have undergone transplantation have reached 20 years of age without major complications. The main risk after transplantation is recurrence of the nephrotic syndrome. At least half the patients with recurrence have circulating antinephrin antibodies, which probably have a pathogenic role in the recurrence.⁷⁰

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Table 1. Hereditary Proteinu	ıria Syndr	omes.			
	Mode of Inheri-				
Disease*	tance	Locus and Gene	Protein	Mechanism	Clinical Description and Comments
Congenital nephrotic syn- drome of the Finnish type (CNF, or NPHS1; OMIM no. 256300)	AR	19q13.1, <i>NPHS1</i>	Nephrin	Mutations in the slit-diaphragm protein nephrin, leading to malfunction or absence of the slit diaphragm	Usually massive proteinuria in utero, with onset of nephrotic syn- drome within the first weeks of life; placenta weight more than 25% of birth weight, kidney transplantation only curative therapy; milder proteinuria phenotype sometimes observed; resistant to corticosteroid and cyclophosphamide therapy; genetic test commercially available
Corticosteroid-resistant nephrotic syndrome (SRNS, or NPHS2; OMIM no. 604766)	AR	1q25—31, NPHS2	Podocin	Mutations in the slit-diaphragm protein podocin, leading to malfunction or absence of the slit diaphragm	Onset and severity of nephropathy varying from early-onset nephro- sis to mild proteinuria starting in early adulthood, resistance to immunosuppressive corticosteroid therapy, early minimal changes, and focal segmental glomerulosclerosis in later stag- es; genetic test commercially available
Pierson's syndrome (OMIM no. 150325)	AR	3p21, LAMB2	Laminin <i>β</i> 2 chain	Mutations in the adult glomerular basement membrane laminin-11 isoform, leading to abnormalities of podocyte and slit- diaphragm development and function; mechanism leading to nephropathy not completely understood	Onset of nephrosis soon after birth; development of diffuse me- sangial sclerosis and microcoria (fixed narrowing of the pupil)
Nail-patella syndrome (OMIM no. 161200)	AD	9q34.1, <i>LMX1B</i>	LMX1B	Mutations in the LMX1B transcription factor, which regulates podocyte genes encod- ing nephrin, podocin, and CD2-associat- ed protein, as well as COL4A3 and COL4A5 type IV collagen	Variable penetrance; nephrotic syndrome as well as skeletal and nail dysplasias in children
Denys-Drash syndrome (OMIM no. 194080) and Frasier's syn- drome (OMIM no. 136680)	AD	11p13, <i>WT1</i>	WT1	Mutations in the WT1 transcription factor, which regulates a number of podocyte genes; mechanism leading to nephropa- thy not completely understood	Male pseudohermaphroditism combined with progressive glomeru- lopathy, early onset of nephropathy, and end-stage renal disease by 3 years of age in Denys–Drash syndrome; later onset of ne- phropathy in Frasier's syndrome, with development of focal segmental glomerulosclerosis; resistant to any treatment ex- cept kidney transplantation
Focal segmental glomeru- losclerosis (FSGS1; OMIM no. 603278)	AD	19q13, ACTN4	α-Actinin-4	Mutations in actin filament-cross-linking <i>a</i> -actinin-4, leading to abnormalities in podocytes, probably by dysregulation of the foot-process cytoskeleton	Mild proteinuria in adolescence or early adulthood; slow progres- sion to focal segmental sclerosis and end-stage renal disease in adulthood
Focal segmental glomeru- losclerosis (FSGS2; OMIM no. 603965)	AD	11q21–22, <i>TRPC6</i>	TRPC6	Mutations in TRPC6, a calcium-permeable cation channel, leading to abnormal podocyte function; mechanism leading to nephropathy not completely understood	Proteinuria in adolescence or early adulthood; progression to focal segmental glomerulosclerosis and end-stage renal disease in adulthood
* Short forms of the disease AR denotes autosomal rece	and the co	orresponding Online	Mendelian I	nheritance in Man (OMIM) numbers are give	n in parentheses.

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Figure 6. Glomerular Phenotype in a Control Subject and in a Patient with the Fin-Major Nephrin Mutation and the Congenital Nephrotic Syndrome of the Finnish Type (CNF).

Panel A shows scanning electron micrographs of podocytes (Ps) on glomerular capillaries of the normal human kidney, with long processes that branch into well-organized, interdigitating foot processes (FP) (inset). The scale bars represent 5 μ m in Panel A and 1 μ m in the inset. As shown in Panel B, the podocytes in a patient with CNF are flattened, with only a few, wide foot processes. The scale bar represents $1 \, \mu$ m. Panel C shows a transmission electron micrograph of a cross section of a normal glomerular capillary. The foot processes are approximately 250 nm wide and are separated by filtration slits (arrows) containing a slit diaphragm. The scale bar represents 200 nm. As shown in Panel D, the flattened and fused (effaced) foot processes of a patient with CNF line the glomerular basement membrane, and the filtration slits (arrow) are far apart. The scale bar represents 200 nm. Panel E shows the boxed portion of Panel C at a higher magnification of Panel C: regular filtration slits (arrows) approximately 40 nm wide are bridged by a thin slit diaphragm. The scale bar represents 100 nm. Panel F shows the boxed portion of Panel D at a higher magnification of Panel D; no slit-diaphragm line is visible, and only faint fuzzy material can be seen in a narrow and elongated filtration slit (arrow). The scale bar represents 100 nm. Panel G shows a tomogram of a typical filtration slit in a glomerulus of a patient with CNF. The slit, which is normally about 40 nm wide, is only about 10 nm wide; it has no organized slit-diaphragm structure, but only some short, unorganized strands. M denotes the podocyte surface membrane. The scale bar represents 5 nm. (Panels A through F are modified from Lahdenkari et al.⁵⁸ with the permission of the publisher, and Panel G is modified from Wartiovaara et al.⁵⁹ with the permission of the publisher.)

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CORTICOSTEROID-RESISTANT NEPHROTIC SYNDROME

Familial autosomal recessive corticosteroid-resistant nephrotic syndrome (SRNS, or NPHS2 [OMIM number 604766]) is characterized by the onset of proteinuria in early childhood, resistance to immunosuppressive therapy, and early progression to minimal-change disease and focal segmental glomerulosclerosis. The cause of the disease is a mutation in the NPHS2 gene for podocin.⁴⁶ NPHS2 mutations have also been detected in sporadic cases of corticosteroid-resistant nephrotic syndrome, in some cases of congenital nephrotic syndrome, and in familial late-onset focal segmental glomerulosclerosis.71-74 Digenic inheritance of NPHS1 and NPHS2 mutations, resulting in a "triallelic hit," appears to modify the phenotype from that of CNF to that of focal segmental glomerulosclerosis.67 All forms of nephropathy caused by NPHS2 mutations are resistant to corticosteroid therapy.73,75,76

Because podocin interacts with nephrin, CD2AP, and the Neph family of proteins and enhances nephrin signaling, the abnormality underlying NPHS2 nephropathy probably involves defective slit-diaphragm function.^{29,38,48,77} Mutations may cause absence of podocin, mistargeting of nephrin into the filtration slit, or compromised signaling.78 More than 30 mutations in the NPHS2 gene have been reported.72-74 Most are located in the region encoding the C-terminal domain of the protein, suggesting a functional role for this domain. Patients with frameshift or truncation mutations have an early onset of disease, whereas many patients with missense mutations have lateonset nephropathy. The most common mutation, R138Q, is likely to be due to a founder effect in northern Europe. The podocin variant R229Q, which is found in about 4 percent of the European population, is associated with an increased risk of microalbuminuria.79

PIERSON'S SYNDROME

Pierson's syndrome (OMIM number 150325) is a rare, lethal, autosomal recessive form of the congenital nephrotic syndrome manifested by diffuse mesangial sclerosis and distinctive ocular anomalies characterized by microcoria (fixed narrowing of the pupil).^{21,80,81} Patients with this glomerular disorder present at birth with massive proteinuria, with rapid progression to renal failure that results in death before the age of two months. The defective gene has been localized to chromosome 3p21, and homozygous or compound heterozygous mutations have been found in the gene for the laminin β 2 chain.²¹ Since this chain is present in the adult glomerular basement membrane laminin-11 isoform (α 5: β 2: γ 1), the renal phenotype is probably due to a malfunction of the glomerular basement membrane. Absence of the laminin β 2 chain in the mouse results in a phenotype similar to that in humans.²⁰

NAIL-PATELLA SYNDROME

The nail-patella syndrome (OMIM number 161200) is an autosomal dominant disease with an incidence of about 1 in 50,000 live births. Its manifestations are symmetric abnormalities of the nails, skeleton, eyes, and kidneys.82 The onset and outcome of the renal disease vary considerably, from renal failure in early childhood to an absence of clinical signs of nephropathy throughout an otherwise normal life. However, the characteristic pathological changes of the glomerular basement membrane, consisting of thickening with splitting and fibrillar collagen deposits, occur in most cases. The disease is caused by lossof-function mutations in LMX1B, a member of the LIM homeodomain family of transcription factors.83-87 LMX1B is expressed in the kidney primarily by podocytes, and it regulates the expression of many crucial podocyte proteins, including nephrin, podocin, CD2AP, and α 3(IV) and α 4(IV) collagen chains.86-88 Dysregulation of these podocyte genes is thought to play a key role in the development of the nephropathy of the nail-patella syndrome.

DENYS-DRASH SYNDROME AND FRASIER'S SYNDROME

The Denys–Drash syndrome (OMIM number 194080) and Frasier's syndrome (OMIM number 136680) are characterized by male pseudohermaphroditism and progressive glomerulopathy.⁸⁹⁻⁹¹ The Denys–Drash syndrome predisposes patients to Wilms' tumor, whereas gonadoblastomas are associated with Frasier's syndrome. In the Denys– Drash syndrome, the nephropathy begins in infancy and progresses to end-stage renal disease by the age of three years. The characteristic glomerular lesion is diffuse mesangial sclerosis. The nephropathy in Frasier's syndrome typically

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begins as focal segmental glomerulosclerosis late in childhood and progresses to end-stage renal disease by the second or third decade of life. However, the clinical manifestations of the two syndromes overlap.92 Both nephropathies are resistant to medical treatment, and kidney transplantation is the only therapeutic alternative.

The Denys-Drash syndrome and Frasier's syndrome are caused by dominant mutations in the Wilms' tumor gene WT1.93,94 Patients with Frasier's syndrome carry mutations in the donor splice site of intron 9 in the gene,94,95 whereas the Denys-Drash syndrome is caused by a number of different mutations distributed along the WT1 gene.96 The WT1 gene encodes a transcription factor that controls the expression of many key podocyte genes, and the nephropathy may be caused by a failure in the regulation of these genes,⁹⁷ although the phenotype of chimeric WT1 mutant mice suggests that the glomerulopathy is mediated by systemic effects of WT1 mutations.98

AUTOSOMAL DOMINANT FOCAL SEGMENTAL GLOMERULOSCLEROSIS

The autosomal dominant forms of focal segmental glomerulosclerosis are a heterogeneous group of inherited diseases characterized by the onset of mild proteinuria during adolescence or early adulthood, with slow progression to segmental glomerulosclerosis and, ultimately, to end-stage renal disease. Two loci have been mapped to chromosomes 19q13 (FSGS1; OMIM number 603278)99 and 11q21-22 (FSGS2; OMIM number 603965).100

Focal segmental glomerulosclerosis type 1 (FSGS1) is caused by mutations in ACTN4, which encodes α -actinin-4.¹⁰¹ α -Actinins are actin filament-cross-linking proteins with different patterns of expression throughout the body. α -Actinin-4 is highly expressed by podocytes, where it cross-links F-actin filaments in the foot processes. Disease-causing mutations increase the affinity of α -actinin-4 for F-actin, which may interfere with the normal assembly and disassembly of actin filaments in the glomerular podocytes.¹⁰² In mice expressing similar high-affinity α -actinin-4 in the podocytes, a phenotype resembling focal segmental glomerulosclerosis develops; mice lacking α -actinin-4 have disrupted podocyte morphology, and endstage renal disease develops in them.^{103,104}

Mutations in the TRPC6 gene, which encodes the transient receptor potential cation channel 6 (TRPC6), were recently identified in families with autosomal dominant FSGS2.105,106 TRPC6 belongs to a family of nonselective cation channels that are involved in the increase in the intracellular calcium concentration after the activation of G-protein-coupled receptors and receptor tyrosine kinases. TRPC6 appears to be associated with the podocyte slit pore, where it is probably involved in slit-diaphragm signaling. A mutant TRPC6 protein can cause abnormally high current amplitudes, which may have a role in the pathogenesis of focal segmental glomerulosclerosis.

CONCLUSIONS

The analysis of several rare genetic disorders in which proteinuria is a prominent feature has led to the identification of proteins required for the development and function of the glomerular filtration barrier. In particular, the new data on these syndromes have yielded insights into the molecular structure of the podocyte slit diaphragm. Progress in the field has also facilitated the classification of hereditary proteinuria disorders, which can vary considerably with regard to age at onset and manifestations. From a clinical point of view, it is important to understand that mutations in the same gene can result in different phenotypes. For this reason, patients with these disorders should undergo genetic testing if possible.

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