Podocyte Differentiation and Hereditary Proteinuria/Nephrotic Syndromes

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Abstract. The study of familial nephrotic syndromes (NS) and the analysis of murine models of glomerular diseases resulted in major progresses in the knowledge of podocyte physiology and pathology. Numerous proteins participating in the composition of the slit diaphragm region have been identified. The importance of several of them (nephrin, podocin, CD2AP, and Nep1) in the maintenance of the glomerular filtration barrier has been demonstrated by the occurrence of massive proteinuria when they are defective. The role of the cytoskeleton has been revealed by the development of proteinuria/NS in patients with ACTN4 mutation and the occurrence of early and severe NS in α-actinin-4-deficient mice. Given the genetic heterogeneity of familial NS and the many other genes to be identified, further insights in the molecular basis of the role of the podocyte in the maintenance of the glomerular filtration barrier may be expected in the near future.

The podocyte is a highly specialized cell in the kidney. Its prominent role in the ultrafiltration of plasma during primary urine formation has been recently highlighted by the characterization of genes coding for podocyte proteins and the demonstration of their involvement in hereditary nephrotic syndromes (NS).

Podocyte Differentiation
As every epithelial cell of the nephron, podocytes stem from precursor mesenchymal cells that are induced and converted to epithelium by the ampullary tips of the branches of the ureteric bud. During nephrogenesis, they undergo dramatic modifications leading from a classical epithelial phenotype at the S-shaped body stage to a very atypical and original phenotype in the mature glomerulus.

Glomerular anlagens are clearly recognized at the S-shaped body stage (1). They seem to derive from the lower limb of the S-body. At this stage, the presumptive podocytes consist of columnar cells with large elongated nuclei. They are polarized: the lateral surfaces are in close contact and connected at their apices by tight junctions (2); the basal surfaces adhere through a continuous cytoplasmic layer to a thin basement membrane made of type IV collagen (α1[IV]2 α2[IV]), laminin 1(α1β1γ1) and 10 (α5β1γ1), perlecan heparan sulfate proteoglycan, and entactin/nidogen (3) and separated from the endothelial basement membrane by mesenchymal matrix. Similar to other epithelial cells, their cytoskeleton includes intermediary filaments of the keratin type (4). They actively proliferate.

With maturation, the cells progressively lose the typical epithelial configuration to become podocytes—unique glomerular visceral epithelial cells that cover the external surface of the capillary tuft. Their voluminous cell body protrudes into the urinary space and lose contact with the neighboring cells. It gives rise to long cytoplasmic processes that run toward the capillaries, divide into pedicels or foot processes, and attach to the glomerular basement membrane (GBM) through adhesion proteins, the α3β1 integrin and the dystroglycan complex. A space, the filtration slit, is present between adjacent pedicels that are derived from different podocytes and is bridged at the basis of pedicels by the slit diaphragm, the only contact between adjacent cells. This slit diaphragm delimits the basal and apical domains of the podocyte, which continues to be a polarized cell. However, the cytoskeleton of keratin has been replaced by vimentin, and the mature podocyte has become unable to replicate. The apical surface of the cell is covered by podocalyxin, a negatively charged, anti-adhesive protein. The GBM has acquired a very special composition. It is basically made of type IV collagen (α3[IV]α4 [IV]α5[IV]), laminin 11 (α5β2γ1), agrin heparan sulfate proteoglycan, and entactin/nidogen (3,5). In addition, the podocyte expresses numerous specific markers, some of them proved to be essential for the maintenance of the size selectivity of the glomerular filtration barrier, as their defect in human or in animal models of human diseases results in abnormal glomerular permeability.

Autosomal Recessive NS
Congenital NS of the Finnish Type
Nephrin, the product of the gene NPHS1 mutated in congenital NS of the Finnish type (CNF), was the first podocyte protein identified through researches on hereditary NS. CNF is frequent in Finland (6) but has also been described in various ethnic groups throughout the world. The disease develops in utero. Infants are premature with a low birth weight for age and a large placenta. Severe NS is present from birth and resistant to steroids or immunosuppressive drugs (6,7). Before the development of active treatment, patients usually died within the...
first 6 mo of life of various complications (7). With supportive treatment, prolonged survival is now possible, but these patients progress to ESRD between 3 and 8 yr of age. Early renal biopsy specimens show mild mesangial hypercellularity and extensive effacement of foot processes. Irregular microcystic dilations of proximal tubules are common but not specific (8).

CNF was initially regarded as a GBM disease, but no mutation was found in eight candidate genes coding for major components of basement membranes. Then, using the positional cloning approach, Kestilä et al. (9) identified a new gene on chromosome 19, mutated in CNF, and named NPHS1. Nephrin, the gene product, is a transmembrane protein of the Ig family of cell adhesion molecules, specifically located at the podocyte slit diaphragm (10). Nphp1 inactivation in mice leads to massive proteinuria, effacement of podocyte foot processes, absence of slit diaphragms, and neonatal death (11). These features indicate that nephrin is a key component of the glomerular filter. They suggest that nephrin molecules, from adjacent podocytes, connect through their Ig-like extracellular domains and form the zipper-like structure, according to the slit diaphragm model presented years ago by Rodewald and Karnovsky (12).

In Finland, two main mutations, Fin-major and Fin-minor, account for >94% of mutations. They are nonsense mutations always associated with a severe disease (13). Recurrence of proteinuria after transplantation, as a result of the development of antinephrin antibodies (14), occurs in 20% of the patients. All of them have Fin-major/Fin-major genotype, which leads to the absence of nephrin in the native kidney. In non-Finnish patients, various types of mutations have been found, some of them having a milder disease progression (13).

**Autosomal Recessive Steroid-Resistant NS**

Autosomal recessive steroid-resistant NS is characterized by an autosomal recessive transmission, onset of proteinuria between 3 mo and 5 yr, resistance to steroid treatment, rapid progression to ESRD, absence of recurrence after renal transplantation, and absence of extraenal disorders. Minimal glomerular changes are observed on early biopsy specimens and FSGS at later stages. Using whole genome analysis, the causative gene, NPHS2, was mapped to 1q25-q31 and identified (15). The protein product, a new glomerular protein, was named podocin.

Podocin is predicted to be an integral membrane protein with a single membrane domain, forming a hairpin-like structure with both ends in the cytosol (15). It belongs to the stomatin family of lipid-raft associated proteins. In the kidney, NPHS2 is exclusively expressed in the podocytes, specifically at the cytoplasmic face of the slit diaphragm (16). Recently, it has been shown that mice that lack podocin develop a severe glomerular disease and die in the first days of life with massive mesangial sclerosis (17). All of these data, as well as the demonstration of its interaction with nephrin and CD2AP (see below) (18), indicate that podocin plays an important role in the maintenance of the slit diaphragm.

All types of NPHS2 mutations have been described. They were not found in all patients demonstrating the genetic heterogeneity of the disease. Two mutations, the R138Q and the R138X, were recurrent, the first one observed in patients originating from Germany or The Netherlands, the second one in families of Israeli-Arab descent. It is interesting that NPHS2 mutations have also been reported in 10 to 33% of sporadic steroid-resistant NS, which represents a frequent cause of ESRD in children (19–21). Rapid screening of these patients for mutation is possible because of the small size of the gene. The identification of mutation allows avoidance of unnecessary treatments, permits the prediction of an absence of recurrence after transplantation, and enables provision of prenat al diagnosis to families at risk. Linkage to NPHS2 has been excluded in familial responsive NS in childhood.

**Schimke Immuno-Osseous Dysplasia**

Schimke immuno-osseous dysplasia, a rare disease, is characterized by the autosomal recessive transmission of spondyloepiphyseal dysplasia and characteristic dysmorphic features, lymphocytopenia and/or T-cell immunodeficiency, and renal dysfunction including proteinuria and NS with development of FSGS and progression to ESRD. The causative gene, SMARCAL1, has been identified (22). Podocyte genes potentially regulated by SMARCAL1, a chromatin remodeling protein, remain to be identified.

**Animal Models of Autosomal Recessive NS**

CD2-associated protein, an adapter protein that anchors CD2 at sites of cell contact, is involved in T-cell activation. Surprising is that CD2AP-knockout mice developed congenital NS and died from renal failure at 6 to 7 wk of age (23). The podocyte expression of CD2AP and the in vitro demonstration of its association with nephrin suggest that it could play a role in the maintenance of the slit diaphragm, perhaps by anchoring the nephrin/podocin complex to the submembranous actin meshwork cytoskeleton (24).

With the use of the gene trapping technology, NEPH1, a novel mouse protein strongly expressed in podocytes and structurally related to nephrin, has been identified. Inactivation of Nephl results in severe congenital NS and perinatal mortality (25). To date, no mutations in the corresponding human homologue genes have been described.

**Autosomal Dominant Proteinuria/NS**

**Familial FSGS**

FSGS, a nonspecific glomerular lesion, may be secondary to various disorders, such as nephron reduction. In some cases, it appears as an idiopathic condition characterized by the presence of isolated proteinuria/NS eventually progressing to renal failure. Recently, familial forms of FSGS have been recognized, most of them with an autosomal dominant inheritance. Evaluation of large families with familial FSGS led to the identification of three loci on chromosomes 1q25–31, 11q22–24, and 19q13 respectively, but several families are not linked to these loci, demonstrating the large genetic heterogeneity of the disease (26–28). The gene located at 19q13 has been identified (28). This gene, ACTN4, encodes α-actinin–4, an actin-binding and cross-linking protein localized to podocytes
in the renal glomerulus, predominantly in the foot processes. In vitro, the FSGS-associated mutations increase the binding of α-actinin to actin filaments (28). The same effect may be expected in vivo, resulting in alteration of the mechanical characteristics of the glomerular podocyte. Recently it has been shown that α-actinin-4 null mice have severe glomerular disease (29).

**Epstein/Fechtner Syndromes**

The association of familial progressive hematuric nephritis and deafness with megathrombocytopenia (plus cataract and leukocyte inclusions in Fechtner syndrome) has long been regarded as Alport syndrome variant, presumably caused by type IV collagen defect. Recently it has been shown that mutations in MYH9, a gene encoding the nonmuscle myosin heavy chain IIA expressed in the kidney, the inner ear, and the platelets, was responsible for these syndromes (30,31). This finding, as well as the demonstration of ACTN4 mutations in familial FSGS and the tight relationships between actin and the slit diaphragm through CD2AP, underlines the importance of the cytoskeleton in the maintenance of podocyte function.

**Nail-Patella Syndrome**

Glomerular symptoms are observed in approximatively 40% of patients with nail-patella syndrome (NPS). The presence of fibrillar collagen within thickened GBM segments suggested that it was a GBM disease. Using different approaches, two groups identified the causative gene (32–34). This gene, LMX1B, encodes a transcription factor involved in dorsoventral patterning of the limb. It is also expressed in the podocyte, from the early stages of differentiation. In the mouse, the protein has been shown to regulate the expression of type IV collagen α4 chain, podocin, and CD2AP, a possible explanation for the occurrence of glomerular disorder in human (35–37). However, no renal symptom and no defect in the expression of these proteins were found in heterozygous mice. No significant changes in their expression were detected in patients with NPS (38).

**Denys-Drash and Frasier Syndromes**

Denys-Drash and Frasier syndromes are caused by mutations in WT1 (Wilms tumor 1), a gene initially reported as a tumor suppressor gene. WT1 encodes a transcription factor, with a zinc finger structure, is normally expressed in podocytes from early steps of nephrogenesis, and is required for early kidney development. The presence of two alternative splicing regions leads to the synthesis of four isoforms with definite and stable proportions (39). In Denys-Drash syndrome, characterized by the association of early-onset glomerulopathy with diffuse mesangial sclerosis, gonadal dysgenesis leading to pseudohypophosphatidosis in males, and a high risk of developing Wilms’ tumor (40–42), dominant negative point mutations affect the zinc fingers of the WT1 protein and, consequently, its binding to DNA (43) and result in abnormal podocyte expression of PAX2 and growth factors PDGF and TGF-β1 (44). Mutations are different in Frasier syndrome, characterized by male pseudohypophosphatidosis with complete sex rever-
sal and streak gonads frequently at the origin of gonadoblastomas, associated with slowly progressive glomerulopathy (45). They are intronic mutations in the second splicing site of the gene (46). They result in the significant reduction of the isoforms containing the sequence KTS (lysine, threonine, serine), demonstrating that a strict equilibrium between the different WT1 isoforms is required for normal renal and testicular development. In the absence of complete data on the in vivo targets of WT1, the precise mechanism leading from WT1 mutation to podocyte dysfunction is still to be determined.

Until recently, these syndromes were regarded as sporadic diseases. However, female patients with WT1 mutations have normal genital development. They now survive to ESRD because of hemodialysis and renal transplantation and are able to become pregnant. They have a 50% risk of transmitting the mutated gene and the disease to their children.

**Maternally Inherited Glomerulopathies**

Recently, a number of cases of glomerulopathies, isolated or associated with extrarenal symptoms (mostly diabetes and/or hearing loss), have been described in patients with mitochondrial cytopathy (47–50). The clinical presentation of the renal disease is unspecific: occurrence of proteinuria at various ages, progressive increase with age, eventual development of NS and FSGS, and variable rate of progression to ESRD. In some cases, increased number of abnormal mitochondria of various shapes and sizes has been observed in podocytes or in individual proximal tubular cells. Most patients with mitochondrial glomerulopathy share the same mutation of mitochondrial trRNALeu gene (A3243G) resulting in the defective synthesis of several mitochondrial proteins. These observations indicate that, in addition to specific defect in podocyte proteins, defect in energy production may result in podocyte dysfunction.

**References**


