Podocyte and hereditary nephrotic syndromes

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Podocytes are highly specialized cells covering the external surface of glomerular capillary tufts. Their voluminous cell bodies protrude into the urinary space and give 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 αβ1 integrin and the dystroglycan complex. The filtration slit between adjacent pedicels derived from different podocytes is bridged at the basis of pedicels by the slit diaphragm (Fig. 1).

The podocyte synthesizes most of collagenic and non-collagenic components of the GBM, and their defects are responsible for hereditary glomerulopathies: Alport syndrome linked to type IV collagen mutations and Pierson disease due to laminin beta2 mutations. In addition, the prominent role of podocytes in plasma ultrafiltration 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, syndromic or nonsyndromic, autosomal recessive or dominant steroid-resistant nephrotic syndrome (SRNS) (Table).

Autosomal recessive nephrotic syndromes

Congenital nephrotic syndrome of the Finnish type and NPHS1 gene

Congenital nephrotic syndrome of the Finnish type (CNF) is frequent in Finland but has also been reported from all over the world. The disease is characterized by massive proteinuria starting in utero. Babies are usually premature with a low birth weight for age and a large placenta weighing over 25% of the baby's birth weight. Severe nephrotic syndrome (NS) present from birth (with serum albumin <10 g/L) is resistant to steroids and immunosuppressive drugs. Before active treatment, patients usually died within the first 6 months of various complications.[1] Prolonged survival is now possible, but patients progress to end-stage renal disease (ESRD) between 3 and 8 years of age. Usually there is no extrarenal symptoms, but 6 of the 70 patients observed in Finland developed muscular dystonia and athetosis.[2] Early renal biopsy specimens
show mild to moderate mesangial cell proliferation, extensive effacement of foot processes, and lack of slit diaphragms (Figs. 2 and 3). Microcystic dilatations of proximal tubules are common but not specific.\textsuperscript{[3]}

After exclusion of 8 candidate genes encoding GBM proteins, the gene NPHS1 responsible for CNF has been localized on chromosome 19 by Kestilä et al.\textsuperscript{[4]} then

### Table. Hereditary nephrotic syndromes

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Fig. 1. Electron micrograph through the peripheral part of normal glomerular capillary walls. The podocyte attaches to the GBM through the foot processes. The thin slit diaphragm is focally seen between adjacent foot processes (uranyl acetate, lead citrate, original magnification × 8000) (insert × 20 000).

Fig. 2. Congenital nephrotic syndrome of Finnish type (2-month-old infant). Glomeruli show moderate mesangial proliferation. Focal dilatation of proximal tubules is associated (PAS, original magnification × 120).

Fig. 3. Congenital nephrotic syndrome of Finnish type (2-month-old infant). Diffuse effacement of foot processes and microvillous transformation. The thin glomerular basement membrane is related to the young age of the patient (electron microscopy, lead citrate and uranyl acetate, original magnification × 9900).

Fig. 4. Familial nephrotic syndrome linked to NPHS2 mutation. Light microscopy showing no glomerular changes on the early biopsy specimen (A) and focal and segmental glomerulosclerosis on the late one (B) (trichrome light green, original magnification × 180).
identified, using the positional cloning technology. It contains 29 exons and, within the kidney, is exclusively expressed in the podocyte. Nephrin, the gene product, is a transmembrane protein of the immunoglobulin family of cell adhesion molecules. Within the kidney, it is specifically located at the podocyte slit diaphragm. Both structure and localization suggested that the large extracellular part of nephrin molecules from adjacent foot processes could interact with each other to form the zipper-like structure of the slit diaphragm described at the ultrastructural level. Recently, the \textit{in vitro} demonstration of homodimeric and heterodimeric interactions between the extracellular domains of nephrin and NEPH1, another slit diaphragm protein, confirmed this hypothesis.

In Finland, two main mutations, Fin-major (2bp deletion leading to stop codon in exon 2) and Fin-minor (nonsense mutation in exon 26) account for over 94% of mutations, suggesting a founder effect. They are truncating mutations always associated with a severe disease. Proteinuria reoccurred in 20% of the patients after transplantation because of the development of anti-nephrin antibodies and not to the reproduction of the primary disease. Nearly all of the patients have Fin-major/Fin-major genotype, which leads to the absence of nephrin in the native kidney. In non-Finnish patients, all types of mutations spanning over the whole gene have been found. Some of them are associated with a relatively slow disease progression. For example, the nonsense R1160X mutation resulting in the loss of the last 81 residues in the intracellular domain of the protein has been found in 13 patients of 11 families, some of them have a severe disease, others with normal renal function at 5 to 19 years of age and a possible response to anti-proteinuric drugs.

The structural importance of nephrin in the formation of the slit diaphragm was confirmed by the occurrence of massive proteinuria, effacement of podocyte foot processes, absence of slit diaphragms, and neonatal death in Nphs1-/-mice.

Autosomal recessive steroid-resistant nephrotic syndrome

Another type of autosomal recessive NS has been identified by Antignac C's group. Typically, the disease, more frequent than CNF, is characterized by early onset of proteinuria, usually before five years of age, resistance to steroid treatment or immunosuppressive drugs, rapid progression to ESRD, absence of recurrence after renal transplantation, and absence of extra-renal manifestations. Recently, however, cardiac anomalies have been detected in 16 of 18 affected children of six consanguineous kindreds of Arab descent.

Renal lesions are not specific: with minimal glomerular changes including effacement of foot processes and absence of slit diaphragms observed in early biopsy specimens and focal segmental glomerulosclerosis at later stages (Fig. 4). The lesions affect a proportion of glomeruli and have a segmental distribution in the glomerular tuft. They are characterized by the obliteration of the capillary lumens by a combination of membranoid material and hyalin deposits. Progression to ESRD is related to the progressive extension of this scarring process.

In a whole genome analysis, one causative gene, NPHS2, was mapped to 1q25-1q31 and identified. The genetic heterogeneity of the disease was demonstrated, some families being not linked to the locus. NPHS2 comprises 8 exons, and the protein product, a new podocyte protein, was named podocin. Podocin is an integral membrane protein with a single membrane domain forming a hairpin-like structure, and with both N- and C-terminal domains in the cytosol. It belongs to the stomatin protein family of lipid-raft associated proteins which have scaffolding function and are involved in mechano-sensation. In the kidney, NPHS2 is exclusively expressed in the podocytes, as soon as there is glomerular formation. The corresponding protein is located specifically at the basis of the foot processes, facing the slit diaphragm.

All types of NPHS2 pathogenic mutations have been found in children with SRNS. According to the different European studies, the detection rate of mutations in the homozygous or compound heterozygous state is 40% to 50% for family cases, confirming genetic heterogeneity. Interestingly, NPHS2 mutations are also detected in 10% to 33% of sporadic cases of SRNS, showing that mutation analysis should be performed in all children with SRNS. Two mutations, R138Q and R138X, are recurrent; the first observed in patients originating from Germany or the Netherlands, and the second in families of Israeli-Arab descent. In China and Japan, NPHS2 mutations have also been detected but they do not appear to be a major cause of SRNS. No pathogenic mutations have been found in patients with familial or sporadic steroid responsive NS or in patients with diffuse mesangial sclerosis.

Age at onset of NS is earlier in patients with two pathogenic NPHS2 mutations (41 months), especially in those with frameshift, truncating and the R138Q missense mutations, than in SRNS patients without detected mutations (108 months). Some genotype-phenotype correlations have been shown that occurrence of NS at birth is often associated with R138Q mutation whereas late onset NS is observed in patients with V180M and R238S mutations, and that proteinuria
develops between the first and third decade of life in carriers of the association of R229Q variant with other NPHS2 mutations. NPHS1-NPHS2 digenic inheritance has been observed in a few patients without any obvious effect on the phenotype. Recurrence of NS after renal transplantation is unlikely in patients with two pathogenic mutations. It has been reported in 5 of 65 patients, with mild clinical impact and favorable response to immunosuppressive treatment in most cases.

The functional consequences of NPHS2 mutations have been studied in HEK cells overexpressing normal podocin or various types of mutants. Compared with the normal plasma membrane localization of wild type protein, most mutants are not targeted to the plasma membrane but are retained in the endoplasmic reticulum or localized in late endosomes. In patients' kidneys, truncated mutations result in the absence or normal localization of podocin whereas most abnormal proteins resulting from missense mutations are retained in the cytoplasm. In all situations, podocin defect is associated with changes in the podocyte distribution of nephrin and other slit diaphragm associated proteins, CD2AP and α-actinin.

Mice lacking podocin develop a severe glomerular disease and die of massive mesangial sclerosis in the first days of life. All of these data as well as the in vitro demonstration of the interaction with nephrin and CD2AP indicate that podocin plays an important role in the maintenance of the slit diaphragm.

**Schimke immuno-osseous dysplasia**

This rare and severe 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 focal segmental glomerulosclerosis and progression to ESRD. The causative gene SMARCAL1, a chromatin remodeling protein, has been identified. Podocyte genes potentially regulated by SMARCAL1 remain to be identified.

**Tetraspanin CD151 disease**

This disease has been found in three patients of Jewish origin. It is characterized by the association of nephrotic syndrome progressing to ESRD, with GBM ultrastructural changes, sensorineural deafness, pretibial epidermolysis, and β-thalassemia minor. It is linked to mutations of CD151 encoding the tetraspanin CD151 (MER2 blood group antigen) that forms stable laminin binding complexes with integrins.

**Steroid-resistant nephrotic syndrome with deafness**

This association of early onset NS progressing to ESRD before the age of 10 years with congenital deafness has been seen in one large Palestinian consanguineous family. A gene locus was identified on chromosome 14q24.2.

**Galloway-Mowat syndrome**

This rare autosomal recessive syndrome is characterized by the association of early onset SRNS, progression to ESRD before the age of 3 years, microcephaly with gyral abnormalities, developmental delay, and hiatus hernia. Various types of glomerular lesions have been described and the genetic defect is still unknown.

**Isolated diffuse mesangial sclerosis**

Diffuse mesangial sclerosis (DMS) is characterized by early onset SRNS, rapid progression to ESRD, and characteristic glomerular changes. It may be isolated or part of the Denys-Drash syndrome. The renal phenotype is similar in the two conditions (see later). Isolated DMS appears to be transmitted as an autosomal recessive trait, with a 25% risk of recurrence of the syndrome in siblings. The defective gene(s) is still unknown. The antenatal detection of affected fetuses is not possible at present, except in the rare forms of antenatal onset of the disease.

**Animal models of autosomal recessive nephrotic syndrome**

CD2-associated protein, an adapter protein that anchors CD2 at sites of cell contact, is involved in T cell activation. Surprisingly, CD2-AP-knock-out mice develop congenital NS and die from renal failure at 6 to 7 weeks of age. The podocyte expression of CD2-AP 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. CD2-AP heterozygous mice have increased susceptibility to glomerular injury and DNA variants were found in 10/45 nephrotic patients, suggesting that CD2-AP haploinsufficiency is also linked to glomerular susceptibility in humans.

NEPH1, a novel mouse protein strongly expressed in podocytes and structurally related to nephrin, has been identified by the gene trapping technology. Inactivation of Nep1 results in severe congenital NS and perinatal mortality. The role of the giant protein FAT1 in the formation of the slit diaphragm has been demonstrated by podocyte abnormalities in mFAT1-/- mice. To date, no mutations in the corresponding
human homologue genes have been described.

**Autosomal dominant proteinuria/nephrotic syndromes**

**Familial focal segmental glomerulosclerosis**

Familial forms of isolated proteinuria/NS which possibly progress to renal failure associated with focal segmental glomerulosclerosis (FSGS) have been recognized, most of them with an autosomal dominant inheritance. Evaluation of large kindreds with familial FSGS may lead to the identification of two genes on chromosomes 11q22-24 and 19q13 respectively, but most families are not linked to these locus demonstrating the large genetic heterogeneity of the disease.

The gene involved in FSGS1 located at 19q13 has been identified.\(^4^0\) 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 process. In vitro, the FSGS-associated mutations increase the binding of α-actinin to actin filaments. The same effect may be expected in vivo, in alteration of the mechanical glomerular podocyte properties, α-actinin-4 null mice have severe glomerular disease.\(^4^0\)

The gene TRPC6 on chromosome 11 has been found to be mutated in FSGS2 patients.\(^4^2,4^3\) This gene encodes a transient receptor potential cation channel expressed in podocytes, which mediates calcium entry into cells. It has been shown that some mutant TRPC6 enhance calcium entry into the cell, in response to agonists, such as angiotensin II, suggesting a mechanism for the progressive alteration of podocyte function.

**Epstein/Fechtner syndromes**

The association of familial progressive hematuric nephritis and deafness with megaloblastic anemia (plus cataract and leukocyte inclusions in Fechtner syndrome) has long been regarded as an Alport syndrome variant, presumably due to collagen IV defect. Recently it has been shown that mutations in MYH9, a gene encoding the non muscle myosin heavy chain IIA expressed in the kidney, the inner ear and the platelets, were responsible for these syndromes.\(^4^4,4^5\)

**Nail-patella syndrome (NPS)**

Glomerular symptoms, proteinuria/NS, are observed in approximately 40% of NPS patients. The presence of fibrillar collagen within thickened GBM segments initially suggested that it was a GBM disease. Using different approaches, two groups identified the causative gene.\(^4^6,4^7\) This gene, LMX1B, is 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, LMX1B regulates the expression of type IV collagen alpha4 and alpha3 chains, and of podocyte proteins podocin and CD2AP, a possible explanation for the occurrence of glomerular disorder in human.\(^4^8-5^0\) However, no significant changes in their expression were detected in NPS patients with severe glomerular disease.\(^5^1\)

**Denys-Drash and Frasier syndromes**

These two syndromes result from mutations in the gene WT1 (Wilms tumor 1) initially reported as a tumor suppressor gene. Actually, WT1 encodes a transcription factor, with a zinc finger structure, required for early kidney and male gonad development.\(^5^2,5^3\) The presence of two alternative splicing regions leads to the synthesis of four isoforms with definite and stable proportions.\(^5^4\) WT1 is normally expressed in podocytes from early steps of nephrogenesis.

Denys-Drash syndrome (DDS) is characterized by the association of early onset glomerulopathy with diffuse mesangial sclerosis.\(^3^5,5^5,5^6\) The initial stage of the disease is characterized by fibrillar increase in mesangial matrix without mesangial cell proliferation associated with podocyte hypertrophy and vacuolation (Fig. 5). The fully developed lesion consists of the combination of thickening of the glomerular basement membranes and massive enlargement of mesangial areas, leading to reduction of the capillary lumens. The mesangial sclerosis eventually contracts the glomerular tuft into a sclerotic mass still surrounded by large and vacuolated podocytes, within a dilated urinary space (Fig. 5). Unilateral or bilateral Wilms tumour may be the first symptom of the disease. However, they are detected after the NS in nearly half of the cases of DDS. Systematic renal echographic survey is imperative in these patients. Male pseudohermaphroditism characterized by ambiguous genitalia or female phenotype with dysgenetic testis or streak gonads has been observed in all except one 46,XY patients. Consequently, the finding of a normal male phenotype usually excludes the diagnosis of DDS. By contrast, all 46,XX children have a normal female phenotype with normal ovaries when information is available. WT1 mutations may be found in young XX females with apparently "isolated DMS", who underwent early binephrectomy (preventing the possible development of Wilms tumour) because of the rapid progression to ESRD.

In DDS patients, dominant negative point mutations affect the zinc fingers of the WT1 protein and, consequently, its DNA binding and result in abnormal podocyte expression of PAX2 and growth factors PDGF and TGFβ1.\(^5^8,5^9\)
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The mutations are different in Frasier syndrome characterized by male pseudohermaphroditism with complete sex reversal and streak gonads frequently at the origin of gonadoblastomas, associated with slowly progressive glomerulopathy.[60] They are intronic mutations in the second splicing site of the gene.[61] They result in the significant reduction of the isoforms containing the KTS sequence (lysine, tryptophane, serine), demonstrating that a strict equilibrium between the different WT1 isoforms is required for normal renal and testicular development.

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 found in patients with mitochondrial cytopathy.[62-65] The clinical presentation of the renal disease is nonspecific: occurrence of proteinuria at various ages, progressive increase with age, possible development of NS and FSGS, and variable rate of progression to ESRD. Glomerular lesions of FSGS are associated with hyaline arteriolar lesions that represent individual myocyte necrosis in afferent arterioles and small arteries.[64] In some cases, the increased number of abnormal mitochondria of various shapes and sizes has been observed in podocytes, or more frequently in proximal tubular cells, despite the absence of tubular symptoms. Interestingly, most patients with mitochondrial glomerulopathy share the same mutation of mitochondrial tRNALeu 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.

In conclusion, the study of familial NS and the analysis of murine models 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, Neph1) 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 occurrence of NS in patients with ACTN4 or MYH9 mutation and the development of early and severe NS in alpha-actinin-4 deficient mice. The importance of precise regulation of podocyte calcium entry has been shown by the implication of TRPC6 in familial NS. Further advances may be expected in the future from the study of additional forms of NS.

Most histological lesions in genetic diseases of podocytes are unspecific. However, the identification of the genetic basis of the disease is useful for the patient: it allows to avoid unnecessary immunosuppressive treatments, to predict the absence of recurrence after transplantation, and to provide prenatal diagnosis to families at risk.

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Fig. 5. Diffuse mesangial sclerosis. Light microscopy showing two different stages of DMS. Early stage showing increase in mesangial matrix and hypertrophy of podocytes (A). Fully developed glomerular lesion characterized by massive enlargement of the mesangial areas associated with retraction of capillary loops. Podocytes are still hypertrophied and vacuolized (B) (PAS, original magnification x250)
References


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