Molecular basis of steroid-resistant nephrotic syndrome

C. Antignac
Inserm U574 and Department of Genetics. Necker Hospital. Paris 5 University. Paris. France.

SUMMARY

The identification of the underlying gene defect in some cases of steroid resistant nephrotic syndrome (SRNS) has recently led to a critical breakthrough in the understanding of the pathogenesis of nephrotic syndromes.

The more severe form of hereditary nephrotic syndromes is the congenital nephrotic syndrome of the Finnish type (CNF). The causative gene, NPHS1, *encodes a novel protein, nephrin which is a transmembrane protein belonging to the immunoglobulin superfamily specifically expressed in the podocyte at the slit diaphragm. Using a positional cloning approach, our group identified a gene,* NPHS2, *involved in a specific entity of familial SRNS characterized by early onset, complete steroid-resistance, rapid progression to ESRD and no recurrence after renal transplantation.* NPHS2 *encodes a novel membrane protein named podocin localized at the cytoplasmic part of the slit diaphragm.*

Familial autosomal dominant cases of primary FSGS have been described in adulthood. Two corresponding genes have been mapped to date, one to 19q13 and the second to 11q21-22. The former has been identified as ACTN4, *the gene encoding the actin-binding protein, α-actinin 4. Other genes involved in the slit-diaphragm or the nephrotic syndrome are CD2-associated protein* (CD2AP), FAT1, WT1, LMX1B, SMARCAL1.

Altogether, these data demonstrate the pivotal role of the podocyte in the development and the maintenance of the glomerular filtration barrier and the crucial role of the genetic factors in the development of SRNS.

Key words: ***Nephrotic syndrome. Slit diaphragm. Genes. Nephrin. Podocin. ACTN4. CD2AP.***

BASES MOLECULARES DEL SÍNDROME NEFRÓTICO RESISTENTE A CORTICOIDES

RESUMEN

La identificación del defecto genético subyacente en algunos casos del síndrome nefrótico córtico resistente (SRNS) ha sido clave en la comprensión de la patogenia del síndrome nefrótico. La forma más severa de síndrome nefrótico hereditario es el síndrome nefrótico congénito de tipo finlandés (CNF). El gen causante, NPHS1, *codifica para una nueva proteína, la nefrina que es una proteína transmembrana que pertenece a la superfamilia de las inmunoglobulinas y se expresa en el podocito a nivel del diafragma de hendidura. Usando el clonaje posicional, nuestro grupo identificó un gen,* NPHS2, *implicado en una entidad específica de SRNS familiar caracterizado por inicio temprano, córticoresistencia, y progresión rápida a IRCT sin recidiva después del trasplante renal.* NPHS2 *codifica para una proteína llamada podocina localizada en la parte citoplásmica del diafragma de hendidura. Se han descrito casos de esclerosis segmentaria y focal autosómica dominante en adultos. Hasta la fecha han sido localizados dos genes causantes de*

esta entidad, uno en 19q13 y el segundo en 11q21-22. El primero se ha identificado como ACTN4, el gen que codifica para la proteína alfa-actinina 4. Otros genes implicados en el diafragma de hendidura y/o el síndrome nefrótico son la CD2-associated protein (CD2AP), FAT1, WT1, LMX1B, SMARCAL1. En conjunto, estos datos demuestran el papel clave del podocito en el desarrollo y mantenimiento de la barrera glomerular de filtración y el papel crucial de los factores genéticos en el desarrollo del SRNS.

Palabras clave: ***Síndrome nefrótico. Diafragma de hendidura. Genes. Nefrina. Podocina. ACTN4. CD2AP.***

Idiopathic nephrotic syndrome (INS) is a clinicopathological entity occurring mainly in children, characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia and edema, contrasting with minimal glomerular changes on kidney sections (with non specific effacement of the podocyte foot processes by electron microcoscopy). Most patients with INS respond to steroid therapy and show a favorable outcome. However, ~20% are steroid resistant with progression to end-stage renal disease (ESRD) occurring in approximately every second case. In these cases, the patients develop focal segmental glomerulosclerosis (FSGS), which ultimately leads to global glomerulosclerosis. In approximately one-third of these cases, nephrotic syndrome recurs almost immediately after renal transplantation, suggesting the existence of a circulating factor responsible for the nephrotic syndrome in these patients. In contrast, the absence of recurrence after transplantation in the other cases suggests the existence of a completely different mechanism underlying nephrotic syndrome, involving the alteration of a structural component of the glomerular filtration barrier. This barrier is a complex structure composed of a basement membrane covered by fenestrated endothelium on the inner surface and highly specialized epithelial cells called podocytes (due to their characteristic interdigitated foot processes) on the outer surface.

Among the steroid-resistant nephrotic syndromes (SRNS), there do exist rare familial cases which do not recur after kidney transplantation. The identification of the underlying gene in some of these cases has recently led to a critical breakthrough in the understanding of the pathogenesis of nephrotic syndromes, and thereby, of the mechanisms of the glomerular filtration, and pinpoints the crucial role of the podocyte.

The more severe form of hereditary nephrotic syndromes is the congenital nephrotic syndrome of the Finnish type (CNF). The causative gene, *NPHS1,* encodes a novel protein, nephrin, which is a transmembrane protein belonging to the immunoglobulin superfamily specifically expressed in the podocyte at the slit diaphragm (Kestila et al., 1998). Search for mutations in the *NPHS1* gene in patients originating from Finland and from other countries showed that two nonsense mutations (Fin-maj and Fin-min) account for over 94% of all mutations in Finland, whereas most mutations found in non-Finnish patients are missense mutations spanning the whole gene (Beltcheva et al., 2001), most of them leading to a defective intracellular nephrin transport (Liu et al., 2001). Interestingly, it has been shown that some mutations can lead to a disease with a variable clinical severity (Koziell et al., 2002).

Using a positional cloning approach, our group identified a gene, *NPHS2* (Boute et al., 2000), involved in a specific entity of familial SRNS characterized by early (usually in the first months of life), complete steroid-resistance, rapid progression to ESRD (around 10 years of age) and no recurrence after renal transplantation. *NPHS2* encodes a novel protein named podocin given its exclusive expression in the podocytes in the kidney, at the cytoplasmic part of the slit diaphragm. Podocin is predicted to be a membrane protein with a single «membrane» domain, probably forming a hairpin with both ends inside the cell and the «bend» embedded in the membrane. *NPHS2* mutations have been found both in familial (~40%) and sporadic cases (10-20%) of SRNS (Caridi et al., 2003; Ruf et al., 2004; Weber et al., 2004). Patients with two pathogenic mutations present with early-onset SRNS and very low incidence of post-transplantation recurrence. In addition, *NPHS2* mutations have also been described in late-onset FSGS, but the affected individuals are usually compound heterozygotes for one *NPHS2* mutation and a nonconservative R229Q amino acid substitution, which occured to be a polymorphism with an allele frequency of ~3.6% in control populations (Tsukaguchi et al., 2002).

Conversely, familial cases called primary FSGS have been described in adulthood, characterized by proteinuria and possibly, but not in all cases, nephrotic syndrome and ESRD, and which are mostly transmitted with an autosomal dominant mode of inheritance. Two corresponding genes have been mapped to date, one to 19q13 and the second to 11q21-22. The former has been identified as *ACTN4*, the gene encoding the actin-binding protein, α-actinin 4 (Kaplan et al., 2000).

Additionally, three knock-out mice lacking (i) CD2-associated protein (CD2AP), initially known as a protein involved in T cell activation and recently shown to be located at the slit diaphragm where it interacts with nephrin; (ii) NEPH1, a novel protein structurally related to nephrin, and (iii) FAT1, a giant protocadherin also located to the slit diaphragm, were found to develop congenital nephrotic syndrome (review in Benzing et al., 2004). No mutations in the human homologues encoding FAT1 or NEPH1 have as yet been identified, whereas a mutation predicted to ablate expression of one *CD2AP* allele has been detected in two human patients with focal segmental glomerulosclerosis, implicating *CD2AP* as a determinant of human susceptibility to glomerular disease (Kim et al., 2003).

It has been recently shown that podocin oligodimerizes and interacts through its C-terminus part, in lipid rafts, with nephrin, CD2AP and NEPH1, and that CD2AP and nephrin associate with the actin cytoskeleton (review in Benzing, 2004). Altogether, these data prove the crucial role of the slit diaphragm components in establishing and maintaining an intact glomerular filter. However, accumulating evidence suggests that slit diaphragm proteins not only serve structural functions setting up a size- and charge-selective filtration barrier, but may also participate in common signaling pathways necessary to maintain the functional integrity of podocytes (ibid). In addition, a specific role for CD2AP in the endocytosis and targeting of proteins to the degradative pathway has been suggested (Kim et al., 2003).

Mutations in transcription factor genes can also lead to the development of nephrotic syndromes such as mutations in *WT1* (Ruf et al., 2004) or in *LMX1B*, leading to the nail-patella syndrome. The recent discovery of the regulation of the nephrin and podocin genes by WT1 and Lmx1b respectively, can help understanding the pathophysiology of the nephrotic syndrome in these various disorders. Along the same line, mutations in the *SMARCAL1* gene cause Schimke immuno-osseous dysplasia, an autosomal recessive disorder which associates spodyloepiphyseal dysplasia, strokes, T-cell immunodeficiency and FSGS (Boerkoel et al., 2002). As *SMARCAL1* encodes a SWI/SF2-related protein involved in chromatin remodeling, it is tempting to speculate that SMARCAL1 regulates expression of podocyte proteins.

Altogether, these data demonstrate the pivotal role of the podocyte in the development and the maintenance of the glomerular filtration barrier. They also show the crucial role of the genetic factors in the development of SRNS and allow explaining the development of one subset of SRNS by the occurrence of structural alterations of the podocyte [or of the glomerular basement membrane, as recently showed by the identification of mutations in the *LAMB2* gene encoding the laminin α2 chain, in patients with nephrotic syndrome and microcoria (Zenker et al., 2004)]. Nevertheless, they also point out genetic heterogeneity in SRNS, emphasizing the need of discovering additional genes involved in these disorders.

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