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Genetic Basis of Nephrotic Syndrome – Review

Obeidová H.[1], Merta M.[1], Reiterová J.[1], Maixnerová D.[1], Štekrová J.[2], Ryšavá R.[1], Tesař V.[1]

[1]Department of Nephrology of the First Faculty of Medicine, Charles University in Prague, and General Teaching Hospital, Czech Republic;
[2]Department of Biology and Medical Genetics of the First Faculty of Medicine, Charles University in Prague and General Teaching Hospital, Czech Republic

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Mailing address: Hana Obeidová, MD., Department of Nephrology of the First faculty of Medicine, Charles University, U nemocnice 2, 120 28 Prague 2, Czech Republic, Phone +420 224 962 570, e-mail: diebo@email.cz

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Abstract: Nephrotic syndrome (NS) is one of the most frequent syndromes characterized namely by heavy proteinuria. Majority of NS occurs as a sporadic form, the incidence of familial cases is from 3 to 5%. Seven genes have been recognized till present, which mutations are responsible for severe forms of NS: NPHS1, NPHS2, ACTN4, CD2AP and WT1, TRPC6, LAMB2. Proteins encoded by these genes (nephrin, podocin, α-actinin-4, an adapter protein anchoring CD2 and others) influence the function of the podocytes. In cases of mutation in NPHS1 gene, causing congenital nephrotic syndrome of the Finnish type (CNF), resistance to steroid therapy occurs regularly and recurrence of proteinuria after renal transplantation is about 20–25%. Mutations in NPHS2 gene lead to autosomal recessive steroid resistant nephrotic syndrome (histologically focal segmental glomerulosclerosis). It was concluded that patients with steroid resistant nephrotic syndrome (SRNS) with homozygous or compound heterozygous mutations in NPHS2 have reduced risk for recurrence of focal segmental glomerulosclerosis (FSGS) in renal transplant (only 8% in comparison with 35% in patients without mutation in NPHS2). A functional polymorphism of NPHS2 gene – R229Q was associated with a late-onset nephrotic syndrome and also with an increased risk of microalbuminuria in the general population. The R229Q variant encodes a protein with lower affinity for binding nephrin. This polymorphism appears to enhance susceptibility to FSGS in association with a second mutant NPHS2 allele. There are also 3 genetic loci connected with autosomal dominant forms of FSGS: ACTN4, TRPC6 and CD2AP (found only in the mice models). These forms of FSGS differ from the recessive form by later-onset and more slowly progressive course of the disease; these mutations seem to be responsible for only a fraction of the autosomal dominant pattern of FSGS.

Nephrotic syndrome is one of the most frequent syndromes characterized by heavy proteinuria, hypalbuminemia, hypercholesterolemia and edemas. Frequent primary causes of NS are minimal change disease (MCD), FSGS and membranous glomerulopathy. The most frequent secondary causes are diabetic nephropathy, lupus nephritis and renal amyloidosis [1]. Majority of NS occurs as a sporadic form. Familial NS was firstly described by Fanconi in 1951. In 1970 several large studies started which evaluated the incidence of familial cases of NS to be from 3 to 5%. The inheritance is autosomal recessive or dominant type, the histopathology mainly shows the focal and segmental glomerulosclerotic lesions and most of the cases of the familial NS are steroid-resistant [2].

NS in children may be congenital (which means present at birth or during the first three months) or it can occur later in life – as so-called late-onset FSGS. High proportion of NS in childhood has a genetic basis. The progress in defining the mutations, their importance for functioning and structure of the glomerular capillary wall and thus their patogenetic role for the leakage of plasma proteins

can help us to show new diagnostic possibilities and to change our approach to the therapy. In this article our current knowledge concerning inborn forms of NS is summarized.

Pathophysiology

The cause of proteinuria in NS is an injury of the function or structure of glomerular capillary wall which is composed of the basement membrane covered at the inner surface by fenestrated endothelium and at the outer surface by highly specialized epithelial cells, the podocytes – characterized by interdigitating foot processes – pedicels. Between foot processes is located a slit diaphragm, which plays the critical role for maintaining the barrier function of glomerular capillary wall. In many acquired and inherited nephropathies, disruption of the glomerular filter is associated with extensive leakage of plasma proteins and a diffuse effacement of the podocyte foot processes, as detected by electron microscopy [3].

The passage of solutes across the glomerular barrier is influenced primarily by the increasing *molecular size* (restriction for molecules larger than 10kDa); disturbances of this mechanism result in nonselective proteinuria. Secondly, it is influenced by *electrostatic forces* imparted by negatively charged cell-surface molecules on the epithelial foot processes formed by capillary sialoproteins, heparansulfates of GBM and podocalyxin; disturbances of this mechanism result in selective proteinuria (leakage of albumin into the glomerular filtrate) [1]. With the disruption of these structural and electrostatic barriers, as seen in many forms of glomerular injury, large quantities of plasma proteins gain access to the glomerular filtrate.

Two main mechanisms are presumed to be responsible for genetic forms of NS. Immunologically, it appears that T cells promote the production of a circulating factor that alters the glomerular permeability of the filtration barrier (the nature of this factor remains elusive); probably in conjunction with the participation of immunoglobulins. This mechanism plays role particularly in SRNS recurring after the transplantation. Second mechanism – genetical, is due to a primary defect in the glomerular filtration barrier and as such it would not be expected to recur after the transplantation (as the kidney donor does not bear the defect) [3].

At present 7 genes, which mutations are responsible for severe forms of NS, are known: *NPHS1, ACTN4, NPHS2, CD2AP, WT1, TRPC6, LAMB2*. Proteins encoded by these genes (nephrin, α-actinin-4, podocin and others) influence the function of the podocytes. New information about the precise arrangement and the interaction of these molecules is essential for the understanding the functional processes occurring on the slit membrane.

A brief review of the different types of disease caused by mutations in these genes follows:

Congenital nephrotic syndrome of the Finnish type (CNF, MIM 256 300)*
CNF gene is situated on chromosome 19q13 – gene NPHS1. The NPHS1 gene spans 26 kb of the genomic DNA and contains 29 exons. The gene product called *nephrin* contains 8 IgG2 motifs, a fibronectin III-like domain and a single transmembrane segment. Nephrin is predominantly expressed in the podocyte, where it localizes to the slit diaphragm. Nephrin is necessary for a normal architecture and function of the GBM. It plays an important role in the regulatory signalling pathways – its intracellular domain contains 6 tyrosine residues which are phosphorylated by Src–family kinases and this phosphorylation modulates the function of nephrin. The intracellular domain of nephrin interacts with another slit diaphragm protein, podocin. The increased interaction with podocin is likely to be secondary to tyrosine phosphorylation of nephrin [4]. In the kidneys of nephrin knock-out mice strain an effacement of pedicels and an absence of a slit diaphragm was observed [5].

The large majority of the congenital NS in Finland (90%) is caused by two NPHS1 mutations: Fin major (the deletion of nucleotides 121 to 122 resulting in a stop codon in exon 2) and Fin minor (encoding a premature termination signal at amino acid 1109, in exon 26). Both mutations lead to the truncated proteins [6].

Most mutations found in the non-Finnish patients are missence mutations; their consequence is a defect in the intracellular transport and retention of the mutant proteins in the endoplasmatic reticulum (possibly as a result of misfolding and unfavoured conformation) [7].

CNF is characterized by an autosomal recessive inheritance. The beginning of the disease is in utero, neonates have a massive proteinuria 20–30 g/day and if they left untreated, they would die from sepsis (secondary immunodeficiency due to severe hypogamaglobulinaemia) rather than a renal failure [8]. Antenatal diagnose can be done only in Finland, where CNF is frequent (incidence 1:10 000 births). It is based on the detection of high concentration of an alpha-fetoprotein. However, prenatal proteinuria and elevated AFP is observed in fetuses both heterozygous and homozygous for NPHS1 defects. Obligatory heterozygotes have no apparent phenotype, though prenatal proteinuria. Prenatal DNA diagnostic (polymerase chain reaction – PCR and direct automatic sequence analysis) is feasible in families with a previously affected child.

The treatment of the disease, known to be resistant to corticosteroids, consists of nephrectomy (usually bilateral to prevent massive protein losses with secondary immunodeficiency and sepsis), dialysis (mostly peritoneal) and renal transplantation. In the recent studies NS recurred in 20 to 25% of kidneys transplanted into Finnish children with CNF. High percentage of these patients displayed anti-glomerular and anti-nephrin antibodies which enabled to identify the extracellular domain of nephrin [9].

**MIM – Mendelian Inheritance of Man*

Focal and segmental glomerulosclerosis (FSGS) AR type (SRN1, MIM 600 995)

Autosomal recessive nephrotic syndrome is caused by a mutation in gene *NPHS2,* mapped on chromosome 1q25-31 and composed of 8 exons. It encodes a protein named *podocin*, member of the stomatin family, which is one of the most important membrane proteins and is exclusively expressed in the podocytes at the foot processes in the place of anchorage of the slit diaphragm [5]. It connects plasma membrane (nephrin) and the cytoskeleton of the podocytes. It includes one transmembrane domain, short extracellular and long cytoplasmatic domain. Podocin was shown to interact with nephrin in the lipid rafts, and also with CD2AP, an adapter protein anchoring CD2. Thus via stabilizing contacts between podocytes, podocin plays a major role in the structural integrity and functioning of the slit diaphragm, which is the maintenance of a glomerular permselectivity [6].

In 2/3 of cases mutations in NPHS2 are homozygous. A large number of mutations has been described till now; the most frequent being 419delG mutation and two missence mutations (R138Q, L169P).

Mutations in NPHS2 gene lead to NS resistant to corticosteroid therapy (also to the treatment with alkylating agents or cyclosporine). It remains one of the most intractable causes of an end-stage renal disease (ESRD) in the first two decades of life. Between 6–21% of all children with sporadic SRNS have mutations in NPHS2 gene [10,12]. It was concluded that patients with SRNS with homozygous or compound heterozygous mutations in NPHS2 have a reduced risk for recurrence of FSGS in a renal transplant (only 8% in comparison with 35% in patients without mutation in NPHS2); immunosuppressive therapy should be reduced at minimum or even discontinued. For this reason it could be recommended to perform mutational analysis of NPHS2 in all children with idiopathic sporadic SRNS and in all children with familial SRNS [10]. However, the detection of this mutation is currently performed only in the context of experimental studies by direct sequencing and denaturing high-performance liquid chromatography (DHPLC).

Recently a functional polymorphism of NPHS2 gene – R229Q (exon 5, G-A transition at nucleotide 755, Arg-Gln substitution in codon 229) – was described. This form is associated with the *late-onset nephrotic syndrome*. It is also associated with an increased risk of microalbuminuria in the general population. (The presence of the R229Q allele is associated with a 2,77-fold increased risk of development of microalbuminuria) [9]. Allele R229Q is present in approximately 3,6% of the western population. The R229Q variant encodes a protein with lower affinity for binding nephrin [11]. This polymorphism appears to enhance susceptibility to FSGS in association with a second mutant NPHS2 allele. So far no study showing late consequences of R229Q heterozygosity has been performed.

Weber et al. [12] conducted a mutational analysis of the gene NPHS2 in 338 patients from 272 families with steroid-resistant NS (SRNS), 81 families with

autosomal recessive SRNS and 172 patients with sporadic SRNS. It was shown that patients with 2 pathogenetic mutations (especially in cases of mutations leading to truncated protein, frame shift mutation or R138Q mutation) are connected with a very early onset form of steroid resistant NS and the recurrence after transplantation is very low (1/32).

Tsakaguchi et al. [13] studied 30 families with primary FSGS (verified by histological diagnosis from a renal biopsy). Mean age of the disease onset was 21.8 years. Other studied subgroup consisted of 91 adults with sporadic primary FSGS. The direct sequencing disclosed the fact, that all affected individuals in a large family were compound heterozygotes for two independent missense substitutions – *R229Q* (exon 5, G-A transition at nucleotide 755, predicting an Arg-Gln substitution at codon 229, which was detected by a loss of the ClaI digestion site) and missence mutation *R291W* (exon 7, C-T transition in nucleotide 941, Arg-Trp substitution at codon 291, which creates a new PflMI digestion site in exon 7). This mutation has been previously described in early-onset FSGS.

Aucella et al. [14] studied 33 patients with sporadic 'adult-onset' FSGS verified by a renal biopsy. Glomerular filtration rate (GFR) was in the normal range in 19 subjects and 14 patients had a variable degree of a renal failure. Families presenting with a clear familial inheritance for proteinuria or other congenital nephrotic syndrome were excluded. The whole coding region, all intron/exon boundaries and flanking intronic regions of NPHS2 gene and the exon 8, i.e. hot-spot mutations of the ACTN4 gene, were analyzed in all patients. The analysis identified 4 already described and 2 new polymorphisms of NPHS2 gene. The R229Q allele was identified in 3/33 patients and in 7/124 controls, accounting for an allelic frequency of 0.045 and 0.028, respectively. The influence of this polymorphism is not known at the moment. The new intronic polymorphism IVS7-54C>T was also found in the exon 8 of the ACTN4 gene.

Autosomal dominant forms of FSGS

Autosomal dominant forms of FSGS differ from the recessive form by later-onset and more slowly progressive course of the disease. Three genetic loci have been identified at the moment, but they seem to be responsible for only a fraction of the autosomal dominant pattern of FSGS.

Mutation in ACTN4 gene encoding the protein alpha actinin 4, were found on chromosome 19q13 and they are associated with the development of FSGS 1 (MIM 603 278). The penetrance of ACTN4 associated disease is high but lower than 100%. ACTN4 is one of four actinin genes. These genes encode highly homologous proteins, biochemically similar (except the calcium sensitivity of a C terminal part). Alpha actinin 4 is a homodimer measuring approximately 100kD. It is the only actinin significantly expressed in the human glomerulus. All the known mutations are missence, increasing the affinity of the protein to filamentous actinin. They affect the mechanical properties of actin gels and via this mechanism they

change the mechanical properties of the podocyte. Mutations of ACTN4 are rarer than NPHS1 and NPHS2 associated nephropathies.

Winn et al. found one family with an autosomal dominant disease mapped to chromosome 11 (11q21) (FSGS2, MIM 603965). The gene encodes TRCP6, a member of the transient receptor potential (TRP) family of non-selective cation channels. It remains unclear how the exaggerated calcium response triggered by TRCP6 translates into FSGS [15, 16].

CD2AP gene causing AD FSGS was found only in the mice models (FSGS3, MIM 607832). CD2AP gene encodes a protein of 80kD, which localizes to the slit diaphragm and directly interacts with C-terminal portion of nephrin.

Diffuse mesangial sclerosis

Diffuse mesangial sclerosis shares similar clinical signs with CNF (because of its early onset) but differs by its rapid progression to ESRD and by the characteristic pattern of glomerular involvement. The proteinuria occurs in the childhood (1–2 years) and the disease progresses rapidly (at the age of about 3 years) to the end-stage of kidney failure. The exact mutation is unknown and the inheritance is autosomal recessive. This form is steroid-resistant, without recurrence after renal transplantation. Antenatal diagnosis is done by the identification of high concentrations of AFP (alpha-fetoprotein) or is based on an ultrasonographic image of hyperechogenic kidney. Isolated defects like nystagmus, mental retardation or microcephalia were found in many patients. Mutations in WT1 gene were also found in isolated cases of diffuse mesangial sclerosis.

Table 1 – Summary of genes responsible for inherited NS

Disease	Gene + localisation	Protein	Inheri-tance	Age of onset	Anomalies	Recurrence after transplantation
CNF	NPHS1 19q13.1	nephrin	AR	Prenatal, Early childhood		20–25%
SRN1	NPHS2 1q25-31	podocin	AR	Childhood, Early adulthood		8% ?
FSGS1	ACTN4 19q13	α4actinin	AD	Early adulthood		
FSGS2	TRPC6 11q21-22	TRPC6	AD	Adulthood		
FSGS3 (mice)	CD2AP 6p12	CD2AP	AD	?		
Frasier sy Denys Drash	WT1 11p13	Transcription factor		Early childhood	Male pseudo-hermafroditism + Wilms tumour	
Pierson sy	LAMB2 3p14-22	laminin β2 chain	AR	Prenatal	Eye abnormalities -microcoria	

Syndromic disease

Podocyte diseases are also found as a part of inherited syndromes. Most frequently they are associated with WT1 mutation. WT1 is a transcription factor, playing role in the development of Wilms tumor. Wilms tumor is an embryonic tumor, which develops through the defects of differentiation of mesenchymal cell, caused by lost of tumorsupressors genes. This gene mapped to chromosome 11p13

Frasier syndrome and Denys-Drash syndrome are related overlapping syndromes caused by mutation in WT1 gene (Frasier sy in intron 9 of the WT1, Denys-Drash sy in exon 9 of the WT1). Both syndromes are characterized by the development of male pseudohermafroditism and glomerular disease. Other syndromes from this family are nail-patella syndrome, Charcot-Marie Tooth or Galoway-Mowat syndrome.

At this point we could mention also Pierson syndrome, caused by mutation in LAMB2 gene encoding the laminin ß2 chain (expressed at the glomerular and arterial basement membranes, lens capsule, retina, basal lamina of intraocular smooth muscles as well as at the basal lamina of the neuromuscular synapse). This syndrome has an autosomal recessive inheritance and manifests by congenital nephrotic syndrome and peculiar eye abnormalities. Its histopathological presentation is diffuse mesangial sclerosis [17].

Discussion

Taking in consideration our current knowledge of the genetic basis of NS several essential questions remain unanswered and some clinically relevant issues deserve to be discussed.

How could be new information on the genetic basis of NS exploited in the clinical practice?

Better understanding of the mechanism of proteinuria in different genetically based proteinuric diseases should help us to refine our diagnostic procedures and also to improve our therapeutic options. In CNF the resistance to steroid therapy is a rule and recurrence of proteinuria after renal transplantation is high (20–25%). Analysis of the antiglomerular and antinephrin antibodies, found in high percentage in this setting, could be helpful in attempts to understand the pathophysiology of such processes.

While autosomal recessive type of FSGS rarely recurs following transplantation, the sporadic variety of FSGS is associated with a 30% recurrence rate. Patients with FSGS, who have homozygous or complex heterozygous podocin mutation, have very low recurrence rate. In the patients with sporadic FSGS the more complex and multifactorial etiology accounts for the recurrence of FSGS – one of the most interesting pathophysiologic factors is a circulating permeability plasma factor (PF). PF is presumed to be present in the circulation of FSGS patients and could reproduce glomerular injury when transferred into the normal host.

Plasmapheresis and immunoadsorption were used successfully in inducing remission of proteinuria in few patients, presumably by the removal of the causative circulating PF. To identify the PF plasma obtained from the plasmapheresis of patients with recurrent FSGS was used. PF has a molecular weight between 30 and 100 kDa and its identity remains obscure [18].

There is also a novel pathway of injury in FSGS. Transmembrane protein CD 80, normally expressed on the surface of B-cell, function in podocytes as an inducible modifier of glomerular permselectivity. CD80 was upregulated on podocytes found in a number of the proteinuric states including the nephrotic syndrome associated with nephrin knock-out mice, drug-induced proteinuria, and immunologically mediated glomerular disease. This linkage between an innate immune response and a gene mutation regulating a slit-diaphragm component may represent a novel scheme for understanding the pathogenesis of recurrent FSGS in some patients [18].

How the new insights in the pathogenesis of genetically based FSGS can influence or change our approach toward renal transplantations in these patients?
Many studies dealing with this topic left some of the clinical observations unclear. Patrakka et al. [9] in the study with 45 transplanted patients with CNF reported a recurrence of proteinuria in 9 of 45; all 9 patients had Fin-major/Fin-major genotype which leads to the absence of nephrin in a native kidney. Antinephrin antibodies were detected in 4/9 patients. It is supposed that in the absence of nephrin, the fetus does not develop tolerance against the protein and following the transplantation an immune response to nephrin, expressed on the graft, develops. The recurrence of proteinuria was observed 2-48 months after the first transplantation. In 3 patients proteinuria occurred during the 1st month after renal transplantation, suggesting the pathogenic role of preformed anti-nephrin antibodies. Therapy with cyclophosphamide, methylprednisolone pulses (and in some cases plasmapheresis) was effective in 7/15 episodes of recurrent nephrotic syndrome.

Ruf et al. [10] in a study with 244 patients (44/244 being after the renal transplantation) proved that children with SRNS caused by homozygous or compound heterozygous mutation in NPHS2 have low-risk of recurrence of FSGS after the transplantation (8% in against 35% in patients without mutation in NPHS2).

In contrast Billing et al. [19] showed in his cohort of 6 patients with SRNS (due to homozygous or heterozygous NPHS2 mutations) an early recurrence of proteinuria after the renal transplantation. This phenomenon remains unexplained due to the fact that proteinuria in all except one patient responded well to increased immunosuppression, whereas their initial SRNS did not. The prompt response to an increased immunosuppression suggests an immunologically mediated glomerular disease; the exact mechanism remains to be clarified.

Niaudet et al. [6] did a study involving 32 transplanted people with two NPHS2 mutations; the recurrence of proteinuria (with histological pattern of FSGS in a renal biopsy) was observed only in 1 case, 2 years after the transplantation. This patient had a (homozygous) mutation R138Q, whilst his mother – living kidney donor – was heterozygous in this mutation.

Bertelli et al. [20] in his group of 9 transplanted patients who were homozygous or compound heterozygous in R138Q had recurrence in 2 patients 10–300 days after renal transplantation. In both cases proteinuria disappeared after therapy with cyclophosphamide and plasmapheresis. Podocin antibodies were not found in both studies.

The recurrence of proteinuria was detected in patients with single heterozygous mutation in NPHS2. Bertelli et al. described 3 patients with heterozygous missence mutation affecting only 1 allele with recurrence of proteinuria 1 month after the transplantation. Niaudet et al. found in a group of 25 patients with sporadic steroid-resistant form of FSGS with recurrence of proteinuria 3 carriers of a heterozygous mutation, 2 of them bearing concomitantly the above mentioned polymorphism. The pathogenetic role of PF in these patients remains to be confirmed. The importance and significance of heterozygous mutations is yet unknown. Therefore, one should be cautious before considering living donor transplantation when the donor is a parent carrying a heterozygous NPHS2 mutation, as the kidney may be more susceptible for the late development of FSGS and as the donor with one kidney may be in an increased risk of developing FSGS.

Should we change our approach to late-onset NS?
During the last years evidence accumulated in favour of the genetic basis in a non-neglect able proportion of patients with late onset NS. Patients with this genetic form are steroid-resistant, so immunosuppressive therapy is not indicated. In part of these cases the renal transplantation is an appropriated therapy. Incidence of recurrence after the transplantation differs (as mentioned above). In the future our attention should be also focused on the donors, especially in cases of family related transplantation.

What is the influence of the polymorphism R229Q of the NPHS2 gene on a renal disease?
This polymorphism is associated with a higher risk of microalbuminuria and increases the probability of the development of FSGS in the presence of another mutation of NPHS2. It is found in 4% of a normal western population. However there are no studies to date focusing on the late consequences of R229Q heterozygosity on the clinical course of the disease [21].

Conclusion
NS is a one of the most frequent syndromes of childhood which occurs in majority as a sporadic form, the incidence of familial cases is from 3 to 5%. At present 7

genes, which mutations are responsible for severe forms of NS are known. New information concerning the mechanism of proteinuria in different genetically based proteinuric diseases should help us in diagnostic procedures and influence our therapeutic options, especially decrease in corticosteroid therapy and importance of the renal transplantation.

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