Genetics of Idiopathic Nephrotic Syndrome

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Abstract. Nephrotic syndrome (NS) is a pathological entity characterized by massive proteinuria and has diverse etiology. Although it is one of the most common renal diseases in children, the etiological factors responsible for idiopathic NS/FSGS remain largely unknown. Previous studies had implicated a variety of factors including genetic factors, although NS is generally regarded as a sporadic disease. Familial cases of NS have however been reported periodically, and both autosomal dominant and recessive forms have been identified. Studies of familial NS /FSGS have led to the discovery of several genes that are expressed in podocytes and are associated with proteinuria. These discoveries have shifted the focus from glomerular basement membrane (GBM) to recognition of the central role of podocytes in maintaining glomerular perm selectivity and pathogenesis of NS/FSGS. Associations with various genes (NPHS1, ACTN4, NPHS2, WT-1) and linkage to several chromosomal regions (such as 19q13, 11q21, 11q24) have been reported in patients with familial NS/FSGS.
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Nephrotic syndrome (NS) is hypoalbuminemia and edema characterized by massive proteinuria. The annual incidence in children has been estimated to be 2.0 to 2.7 per 100.000 in USA with a cumulative prevalence of 16 per 100.000. Geographic or ethnic differences have been reported with a 6-fold greater incidence in Asian than in European children.[1-4] NS can be classified as either primary (also called idiopathic) or secondary and can occur at any age. In children, primary forms of NS are mainly seen in association with minimal change disease, which is usually associated with good prognosis. However, other pathologic entities may present with primary NS including mesagioproliferative glomerulonephritis and focal segmental glomerulosclerosis (FSGS).[5] FSGS is a significant cause of end-stage renal disease (ESRD), comprising up to 5% of adults and 15-20% of children with ESRD.[5, 6] Although it is a common renal disease in children, the etiological factors are largely unclear. Previous studies had implicated a variety of factors including genetic factors, although NS is generally regarded as a sporadic disease. Familial cases of NS is generally thought of as a sporadic condition, but genetic factors appear to be important in its pathogenesis, with both autosomal dominant and recessive forms identified. These familial cases have helped our understanding of the genetics and pathobiology of proteinuria. Lately, associations with various genes and linkage to several chromosomal regions have been reported in patients with familial NS/FSGS.[7] An even larger number of genes have been associated with nephrosis and proteinuria in animals. It is now increasingly being recognized that some of the genes identified for familial cases of NS/FSGS may also be important in the more common, so called, "sporadic" versions of the disease. The following sections present a brief overview of the current state of our knowledge of the various genes identified for their association with the phenotype of NS/FSGS and their role in current management practices.

Abbreviations

FSGS	=	Focal segmental glomerulo sclerosis
NPHS1	=	nephrin
NPHS2	=	podocin
ACTN4	=	Alpha actinin 4
WT1	=	Wilms' Tumor 1
CD2AP	=	CD2 associated protein
TRPC6	=	Transient receptor potential 6

Glomerular Structure and Genes Associated with Primary NS/FSGS

An understanding of the glomerular filtration barrier is required to appreciate the recent developments in genetics of NS/FSGS. The glomerular filtration barrier is the target of injury in these syndromes and separates the blood from urinary spaces. It selectively permits the ultra-filtration of water and solutes while preventing the leakage from the vasculature of large molecules, with a molecular weight greater than 40,000Dalton (40 kDa), such as albumin and clotting factors. This process is also called glomerular perm selectivity. The filtration barrier consists of three main components: 1) glomerular visceral

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epithelial cells (podocytes); 2) glomerular basement membrane (GBM) and 3) the fenestrated endothelial cells. The podocytes cover the outer surfaces of a thin GBM, measuring about 0.3 μm, which in turn rests on the fenestrated glomerular capillary endothelial cells that lie on the other side. For the last 3-4 decades, the GBM was thought to play a major role in the pathogenesis of proteinuria. However, in the last 5-7 yrs, studies of familial NS/FSGS have led to the discovery of several genes that are expressed in podocytes and are associated with proteinuria.[7, 8] These discoveries have shifted the focus from glomerular basement membrane (GBM) to podocytes in pathogenesis of NS/FSGS.[7, 9] Briefly, podocytes are specialized epithelia with a cell body and several foot processes that are in contact with each other through the inter-podocyte connection, called slit-diaphragm (SD). SD is now being recognized as a well-differentiated structure with unique functions and has an electron dense zipper-like structure composed of several components. The extra-cellular components, including nephrin, are connected through other specialized structures with in the cell (*i.e.*, podocin, CD2AP) to the main cell body.[7, 8] Fig. 1 presents a diagrammatic representation of some of the podocyte components identified for their association with NS/FSGS in humans. There have been several excellent reviews published lately on various genes associated with NS/FSGS, and additional information can also be obtained by querying the On-line Mendelian Inheritance in Man (OMIM) database web sites (www.ncbi.nih.gov).[7-10] The inheritance patterns associated with the different NS/FSGS genes can be either autonomic dominant or recessive. Several of these genes are further described in the section below:

GENES WITH PRIMARILY AUTOSOMAL RECESSIVE INHERITANCE

Nephrin (NPHS1)

The NPHS1 gene is located on chromosome 19q13 and was the first gene to be associated with human nephrotic syndrome.[7, 11] The gene encodes a large 136 kDa protein called nephrin which was also the first molecule to be identified that specifically localizes to the SD. Nephrin is an extensively N-glycosylated transmembrane protein with a large extracellular portion with eight immunoglobulin-like domains. It is proposed that neighbouring nephrin molecules extend towards each other from adjacent foot-processes and interact through homophilic dimerisation to form a zipper-like structure. This structure of SD is also seen in electron micrographs.[11] It is now becoming apparent that, besides forming homo-dimers with neighboring, nephrin also interacts with other SD molecules, like podocin, to form raft like structures and may play a role in cell signaling. Recently, at least three additional nephrin-like homologues: Neph1, Neph2 and Neph3 have also been identified in rodent models and have properties like nephrin.[12, 13] For example, Neph1 is also localized to the SD and forms homodimers with itself as well as heterodimers with nephrin.[12] The loss of Neph1 leads to foot process effacement and early postnatal death in mice, which is similar although less severe than the disease seen in nephrin knockout mice. In addition, all three Neph-like proteins can interact through their cytoplasmic tails with podocin (discussed later). Mutations in nephrin were initially found in patients with the autonomic recessive congenital nephritic syndrome of Finnish type (CNF) in 1998 by positional cloning.[11] CNF is a severe form of nephrosis with onset of proteinuria generally in neonatal (or even prenatal period) and is characterized by a rapid progress to renal failure. Renal histology in these patients is characterized by micro-cystic glomeruli, progressive glomerular sclerosis with extensive foot process effacement.[14] Two main mutations termed, Fin major and Fin minor, were initially proposed to account for majority of the mutations causing CNF in the Finnish population.[11,15] However, over the last several years a number of mutations, which can be present anywhere in the gene, have been reported from patients all over the world. Besides the homozygous mutations in nephrin that are associated with the relatively rare CNF, nephrin mutations or single nucleotide polymorphisms (SNP) may also have a role in the more common and less severe forms of NS.[16] Heterozygous mutations as well as mutations in conjunction with podocin gene mutations have been associated with NS that has its onset later on in childhood.[17-19] Studies are currently in progress to identify the association of SNPs in nephrin and other NS/FSGS genes as well as nephrin homologues with various common renal diseases characterized by proteinuria, such as diabetic nephropathy.

Podocin (NPHS2)

The NPHS2 gene lies in the chromosome 1q25-q31 region and was identified as the causative gene in steroid-resistant nephrotic syndrome of childhood onset in the year 2000.[20] The NPHS2 gene encodes a 383-amino acid protein that is called podocin. Podocin is a hairpin like protein of approximately 42 kD. The 3-prime untranslated region contains an atypical polyadenylation signal situated upstream of the poly(A) tail. Podocin is an integral membrane protein with 1 transmembrane domain and a C-terminal cytoplasmic tail.[20-22] Database comparisons showed that podocin was an unusual protein which was unlike any other known protein. It has a region of extensive similarity only between its central region and proteins of the band-7-stomatin family. The strongest homology was found with human stomatin (47% identity over 253 amino acids) and *C. elegans* MEC-2 protein (44% identity over 275 amino acids). A 2-kb RNA transcript of podocin is strongly expressed in human fetal and adult kidney, where it is seen almost exclusively in the glomeruli with no signal being observed in other tissues. Within the glomeruli the RNA

expression is restricted to the podocytes. In developing kidney, podocin is expressed in a time-dependent fashion with no signal detected in the earlier stages of nephron development. The expression increases in the future podocytes in the inferior segment of the S-body.[21, 22]

Podocin is a raft-associated component of the glomerular foot-process membrane where it is localized at the insertion of the SD (Fig. 1).[19] It can form oligomers in the raft where it forms a membrane invagination and recruits nephrin and CD2AP in these microdomains. Podocin is required for nephrin transport to membrane and for podocyte intracellular signalling. The mice-lacking podocin (NPHS2-/-) develops extensive podocyte lesions and proteinuria before birth and then die from uremia after a few days of life. However, these NPHS2-/- mice do not develop FSGS lesions but show extensive effacement of foot process and diffuse mesangial sclerosis with tubular dilatation reminiscent of the pathology seen in CNF.[21] These mice lack not just podocin but also nephrin. Therefore, it seems that the molecular scaffold of the SD assembly disassembles even if one component is removed. This appears to be the case in several proteinuric states, in which the expression of most podocyte components is diminished as the result of foot process effacement.

Mutations in podocin were originally identified in infants with early onset NS /FSGS. Recently, mutations in this gene have also been identified in a much larger cohort of patients and have been reported from all over the world.[20, 23-25] In one study, nine out of 30 families with an autosomal recessive inheritance pattern of a delayed onset of FSGS showed mutations in podocin.[23] NPHS2 mutations were shown to account for a significant part of all nephrotic patients roughly corresponding to a mutation detection rate of 45–55% in families with recessive traits and 8–20% in sporadic NS that included different groups and all the clinical phenotypes in a large European study. Thus, mutations of NPHS2 have now accounted for most of familial NS with recessive inheritance and have been seen in sporadic cases as well. More than 50 NPHS2 mutations have been identified and reported so far. Reported mutations involve the whole length of the gene and determine every kind of alteration, including missense, nonsense, and deletion.[26-29] Thus podocin is turning out to be a major contributor to the genetic burden of NS/FSGS.

GENES WITH PRIMARILY AUTOSOMAL DOMINANT INHERITANCE

CD2-associated protein (CD2AP)

CD2-associated protein (CD2AP) gene is located on chromosome 6p12 and encodes an 80 kDa SH3-domain containing protein that is critical for stabilising the contact between a T cells and antigen-presenting cells.[30] The CD2AP has at its N-terminus, an actin-binding site, and the relative cellular location of this protein is shown in Fig. 1. It is a multifunctional adapter-type molecule which is localized in the cytoplasm and leading edges of cells growing in cell culture. Its structure and colocalization with F-actin suggests it has a role in the dynamic regulation of the actin cytoskeleton. CD2AP is expressed primarily in podocytes at the cytoplasmic face of the SD.[31] Several experiments have shown that CD2AP interacts with nephrin and both are involved with intracellular signalling.[32] The role of CD2AP in NS /FSGS pathogenesis was found almost unexpectedly when CD2AP knock out (KO) mice were found to develop a nephrotic syndrome like disease and die at around 6 weeks of age from renal failure.[30] The kidneys of CD2AP –/– animals (a homozygous state where both parental copies of the gene are knocked out is designated as –/– and heterozygous state as +/–) show several features of NS / FSGS pathology, such as mesangial cell proliferation with extracellular matrix deposition and glomerulosclerosis with extensive foot-process

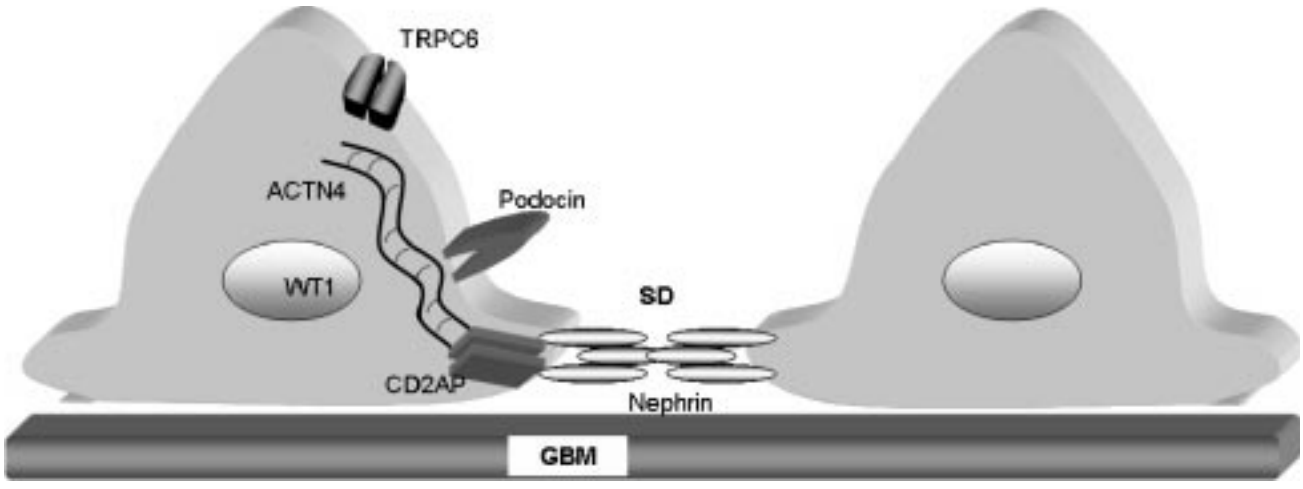

Fig. 1. Podocyte components associated with nephrotic syndrome. SD: slit diaphragm, GBM: glomerular basement membrane. The genes and symbols are described in the text.

effacement. Recently, even the heterozygous knockout (CD2AP +/–) mice were shown to develop an FSGS like glomerular pathology.[33] These animals developed symptoms at a later age of about 9 months of age (vs 6 weeks in homozygous knock out mice). CD2AP +/– heterozygous mice also show reduced concentrations of CD2AP protein. These findings suggest that CD2AP gene defects can cause disease both in homozygous state (autonomic recessive) as well as heterozygous or haplo-insufficiency state (a condition analogous to autonomic dominant inheritance). Based on these mouse findings, a search for mutations in the CD2AP gene in 30 African-Americans with idiopathic FSGS was performed. This study showed that a variant of CD2AP was present in 2 patients that result in aberrant splicing of the molecule.[33] This heterozygous polymorphism was shown to cause reduced CD2AP expression in B-lymphocytes and thus had functional significance. Collectively, these results suggest that CD2AP may be an important in causation of glomerular disease in humans. However, the role of CD2AP in the pathogenesis of human NS / FSGS is not clearly established as only 2 patients with idiopathic FSGS have so far been shown to have the heterozygous CD2AP mutation. More work needs to be done in a much larger number of FSGS patients and probably from different ethnic backgrounds, to firmly establish this association.

Alpha-actinin 4 (ACTN4)

ACTN4 is a component of the actin cytoskeleton that binds to F actin and the gene, like NPHS1 lies on chromosome 19q13.[34, 35] ACTN4 is important in nonmuscle cytoskeletal function and is upregulated early in the course of some animal models of nephrotic syndrome.[36] The identification of mutations in ACTN4 in patients with an autosomal dominant form of FSGS leads to the initial realization of the importance of the cytoskeleton in glomerular function. The linkage of a familial form of FSGS to this locus was initially reported in a large family from Oklahoma, USA.[34] Subsequently, two additional families were also linked to this chromosome, and missense mutations of ACTN4 were identified in each of these 3 families. The mutations were shown to induce a greater binding of ACTN4 with F-actin, suggesting that perhaps the mutations alter the mechanical characteristics of the glomerular podocyte.[35] ACTN4 is widely expressed throughout the body and is highly expressed in the glomerular podocyte. It is unclear why the patients with mutations in ACTN4 should develop a disease that is restricted only to the glomerulus even though ACTN4 has a wide expression in the body. Further studies have shown that homozygous knockout mice also develop advanced glomerular disease with proteinuria, show blebs in the GBM and foot process effacement by 10 weeks of age.[37] In a different set of experiments,[38] over-expressions of the human mutation in mice also lead to proteinuria and glomerular lesions that resembled FSGS. Thus, it is interesting that both loss and gain of function mutations in ACTN4 can lead to glomerular disease and suggests that subtle inherited and acquired changes in this molecule may be involved in human glomerular disease. However, like CD2AP more work is required to establish a role for ACTN4 in human NS/FSGS, as very few patients have been reported with mutation in this gene.

Wilms' Tumor Gene (WT1)

The Wilms' tumor gene (WT1) is located on chromosome 11p13 and was originally discovered as a tumor suppressor gene inactivated in a subset of Wilms' tumors.[39] WT1 gene is composed of 10 exons and encodes a protein with four zinc fingers. WT1 has several different isoforms. Two major alternative splice sites in WT1 generate four major WT1 isoforms. Further, posttranscriptional modifications and RNA editing can increase the number of known WT1 protein isoforms to at least 24.[40] Regulated expression of WT1 is one of the major requirements for normal renal and genital development but mechanisms that regulate WT1 expression are still unclear. WT1 is expressed in podocytes from the capillary loop stage of development onwards and WT1 is required for podocyte function. Mutations in WT1 are typically seen in sporadic Wilms' tumors and occur frequently in association with Frasier and Denys-Drash syndromes.[41-43] These two syndromes are characterized by gonadal dysfunction and progressive nephropathy with FSGS or diffuse mesangial sclerosis with onset in early childhood. In addition, isolated diffuse mesangial sclerosis patients have been shown to have WT1 mutations especially

TABLE 1. Summary of Genes Associated with Humans Idiopathic NS/FSGS

Inheritance	Syndrome (Gene)	Symbol	Chromosome	Age of onset	Pathology
Autosomal recessive					
	CNF (Nephrin)	NPHS1	19q13	Congenital	FSGS / cyst-like
	Podocin	NPHS2	1q25	Childhood	FSGS
	SSNS	?	2p12-p13.2	Childhood	FSGS
Autosomal dominant					
	FSGS1	ACTN4	19q13	Adulthood	FSGS
	FSGS2	TRPC6	11q21	Adulthood	FSGS
		CD2AP	6p12	Adulthood	FSGS (sporadic cases)
	FSGSAS	??	11q24	Adulthood	FSGS
	Denys Drash, Frasier	WT1	11p13	Congenital	DMS / cyst-like

in exons 8 and 9, involving the zinc finger domain.[40] Mice experiments have also confirmed these observations. Mice in which the WT1 gene is homozygously deleted fail to develop kidneys and gonads, but heterozygote animals with a deletion of the third zinc finger develop mesangial sclerosis and show podocyte foot process fusion and proteinuria.[44] The expression of a number of podocyte proteins including nephrin and podocalyxin are also reduced in heterozygote animal. Mice models also show defects in glomerular capillary formation suggesting that WT1 may regulate vascular factors in the podocyte. Recently several single nucleotide polymorphisms (SNPs) in WT1 gene were associated with FSGS in the high-risk group of African Americans.[40] However, further studies are needed to confirm this association and to identify whether these SNPs may mediate NS/FSGS pathogenesis by altering WT1 function.

Transient Receptor Potential 6 (TRPC6)

The TRPC6 gene is located on chromosome 11q24 and has been recently linked to human NS/FSGS.[45] This ion channel is expressed in podocytes and has been identified as a component of the SD.[46] TRPC6 is a member of the transient receptor potential (TRP) superfamily of cation-selective ion channels. The TRPC subfamily (TRPC1-TRPC7) is a group of calcium-permeable cation channels that are important for the increase in intracellular Ca2+ concentration after the engagement of G protein-coupled receptors and receptor tyrosine kinases. According to their primary structure, mammalian TRP proteins are currently classified into six subgroups: TRPC, TRPV, TRPM, TRPP, TRPML, and TRPA. The seven members of the TRPC subfamily (also called classical or canonical TRPs) are structurally related to Drosophila TRP and also to each other (>30% within the first 750–900 amino acids) but differ mainly within the carboxyterminal region. Furthermore, it was shown that TRPC1, TRPC4, and TRPC5 or TRPC3, TRPC6, and TRPC7, respectively, could specifically interact with each other and form homo- and heterotetramers that can interact with a variety of other proteins. TRPC proteins have been shown to be activated or modulated by agonist- and receptor-mediated stimulation of phospholipase C. TRP channels are involed in diverse biological functions such as cell growth, ion homeostasis, mechanosensation and PLC-dependent calcium entry into cells. Calcium as a second messenger affects many of these same cellular functions.

Recently mutations in this gene were reported in a large family with autosomal dominant FSGS hailing from New Zealand.[45] Almost simultaneously, another study reported five families, also with autosomal dominant FSGS, in which the disease segregated with mutations in the gene TRPC6. In the large New Zealand family, a single missense mutation (C335A) in exon 2causing a proline to glutamine substitution at position 112 (P112Q) within the first ankyrin repeat of the TRPC6 protein was found to be responsible for the disease in all the affected individuals. This variant was also shown to be associated with exaggerated calcium signaling *in vitro.* Additional mutations were reported in the second study of the five families.[46] Two of these TRPC6 mutants also had increased calcium signaling. If the calcium signaling is similarly increased *in vivo*, these mutations could thus lead to a gain-of-function activity and increased calcium influx. It is possible that the exaggerated calcium influx conferred by the TRPC6P112Q and other mutations can disrupt glomerular cell function. These mutations of TRPC6 generally cause a delayed onset of disease (usually in 3rd decade or later) and it is unclear, as to why such individuals may be protected for a prolonged period. It is possible that these mutations produce only subtle changes in intracellular function that lead to irreversible cell injury only over a period and may require other renal insults. In addition, podocytes also express several other TRPC channels, including TRPC1, TRPC2 and TRPC5, which may have partial functional redundancy. Thus a complex cellular regulation of calcium homeostasis by TRPC6 is likely required to normal podocyte function, and mutations in this gene or its SNPs may act as modifiers of proteinuric states. These studies convincingly show that TRPC6 channel activity at the SD is essential for proper regulation of podocyte structure and function. In contrast to the previously identified NS/FSGS genes which play a role in cytoskeleton structure or maintenance, TRPC6 is the first calcium-permeable channel that has been implicated in FSGS pathogenesis.

Additional Linkage and Genetics of NS/FSGS in India

Besides the NS/FSGS genes already described, there are several more that have been mapped to different chromosome but not identified.[49-52] We had previously reported a large African American family with linkage to 19q13 region, which does not have mutations in nephrin or ACTN4 genes, suggesting presence of at least one more gene in this region.[49] A new locus on chromosome 2q12 has also been reported for the steroid sensitive NS.[50] It is likely that a similar genetic burden of the disease involving very similar genes and mutations may exist in India as seen in the west. It is also likely that a different set of genes may be operative in the Indian/South Asian context. We recently reported a large family hailing from North India with FSGS like feature and deafness with linkage to 11q22 region that was distinct from the 11q24 region that has been shown to contain the TRPC6 gene.[52] In addition, several familial cases have been observed in pediatric renal clinics in several distinct geographical regions of India (personal communication, A Bagga). Efforts should be made to identify unique familial cases of nephropathy with Indian/south Asian region and appropriate studies linkage and association studies initiated that would give a clearer picture of the genetic epidemiology of this problem in India.

CONCLUSION

The identification of the genetic basis of familial NS / FSGS has significantly advanced our understanding of the mechanisms underlying renal permselectivity and proteinuria. Table 1 summarizes our current understanding of the genetic basis of NS. Many of the genes that were originally identified in rare forms of inherited nephrosis appear to be more broadly involved in the modulation of susceptibility to glomerular injury and disease. The functional model for glomerular permselectivity is emerging following identification of various NS/FSGS genes and involves several interacting molecular components. The large number of genes currently identified (or yet to be identified) helps explain some of the clinical and genetic heterogeneity of NS/FSGS. For example, identification of mutation in a particular gene could explain age at onset of proteinuria, which occurs early in case of nephrin or podocin mutations, and starts later in adulthood in case of ACTN4 or TRPC6 mutations. Further, mutations or polymorphisms in these or other genes may also act as "modifiers" of a clinical course. Current indications for testing of mutations in NS/FSGS genes would include familial occurrence and difficult steroid of therapy-resistant cases where identification of a genetic mutation can help in avoiding further tonic therapy. Also, there is some evidence to suggest that NS/FSGS patients with mutations in podocin may have a lower likelihood of FSGS recurrence post-transplant. It is possible that in very near future the clinically heterogenous syndromes of nephrosis/FSGS may need to be reclassified based on a gene-based classification. Finally, further research on molecular genetics of familial FSGS and NS is needed, as many forms remain to be characterized. This represents an important goal for future studies in informative pedigrees and should be undertaken in the Indian context.

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