

Yet More Ways to Skin a Cat: Nephrin Mutations outside the Neonatal Period

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The earliest descriptions of a genetic basis for the nephrotic syndrome concerned clinical phenotypes of relatively limited variability apparently as a result of classical Mendelian disorders with monogenic inheritance. Paradigmatic was the description by Tryggvason and his colleagues¹ of the genetic basis of the Finnish type of congenital nephrotic syndrome (CNS) caused by autosomal recessive mutations in *NPHS1* (encoding the protein nephrin). Subsequently, Antignac and colleagues² described the gene *NPHS2* (encoding the protein podocin) responsible for an autosomal recessive form of steroid-resistant nephrotic syndrome (SRNS) presenting later in childhood. To date, eight genes expressed in glomerular podocytes have been described, mutations of which are associated with development of the nephrotic syndrome with onset varying from the neonatal period to adulthood.

The discovery of mutations in these same genes outside the clinical phenotypes of SRNS in which they were first described has shed new light on the complexity of the genetic basis of SRNS. Mutations in *NPHS2* have been found in a large number of infants with CNS (more commonly than *NPHS1* mutations in one study³). Genetic heterogeneity of CNS, including triallelic involvement of *NPHS1* and *NPHS2*, has also been described.⁴ Even within the classical age range of SRNS caused by *NPHS2* mutations, nonsense or homozygous p.R138Q mutations have been shown to present earlier than (other) missense mutations.⁵ Now Philippe *et al.*⁶ report in this issue of *JASN* that mutations in *NPHS1* are found in some patients with SRNS presenting outside the neonatal period. As with neonatal presentation of triallelic *NPHS1/NPHS2* mutations,⁴ these individuals manifest histologic patterns quite different from classical CNS.

Why are these mutations now being found outside their original phenotypes? In part, this may be because genotyping of such cases has so far been difficult and expensive, so mutations in a particular gene have generally not been sought in patients “outside” the classical phenotype. Could these extended phenotypes also be due to novel mutations? At least one mutation in *NPHS2* (leading to p.R138Q in podocin) has been described both in CNS^{3,4} and in infantile and childhood-onset SRNS.^{2,4} This is also the case for at least two of the 14 mutations in *NPHS1* reported by Philippe *et al.*,⁶ which have been reported before in the context of classical CNS, in one case (p.R460Q) associated as a heterozygous mutation with neonatal onset of nephrotic syndrome leading to kidney failure at 10 mo of age.⁷ Alternatively, does SRNS appear later in some patients because they are compound heterozygotes with one “mild” mutation in *NPHS1*? Philippe *et al.* present *in vitro* functional evidence for normal trafficking to the plasma membrane and protein–protein interactions of nephrin in most of their missense mutations. At the same time, two of the mutations with some preserved *in vitro* functions were severe enough to lead to kidney failure by 13 yr of age.

Because Philippe *et al.* excluded in advance patients having detectable *NPHS2* or *WT1* mutations, the interesting question of oligogenic involvement of mutations in *NPHS1* and in one or both of these other genes cannot be addressed from their study. A recent report⁸ suggested that bigenic (digenic) mutations in five genes (*WT1*, *NPHS1*, *NPHS2*, *CD2AP*, and *PLCE1*) may account for a substantial number (four of 19) of cases of sporadic childhood-onset SRNS, so undetected digenic mutations could conceivably explain nonclassical phenotypes associated with *NPHS1* mutations. The occurrence of heterozygous *NPHS1* mutations^{3,9} suggests that digenic mutations also may occur in classical presentations of CNS. Clearly, we do not as yet understand the genetic basis for phenotypic variation in SRNS; simple monogenic models are certainly inadequate.

A complex form of inheritance has been well described in other genetic diseases affecting the kidney. Bardet-Biedl syndrome (BBS) is associated with mutations in 12 different genes and shows broad phenotypic variability.¹⁰ Katsanis¹¹ proposed that this variability is in part due to an oligogenic (trialelic) mode of inheritance, based on epistatic interactions of heterozygous (missense) mutations in a BBS-associated gene with homozygous or compound heterozygous mutations in another BBS-associated gene. Mutations in these genes presumably interact because the genes underlie a system (or module¹²) of interacting proteins (in this case, proteins of the sensory cilia and central body in tubule cells). Similarly, because a large number of

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genes are involved in the nephronophthisis (NPHP) spectrum, Hildebrandt and colleagues¹³ investigated and detected cases of oligogenic inheritance in their NPHP patient population. As with BBS, nephronophthisis is characterized by broad phenotypic variation. The protein products of the NPHP-associated genes also interact in the primary cilia of renal tubules.

Given the aforementioned examples of BBS and NPHP, it should hardly be surprising if digenic mechanisms were also operative in at least some cases of SRNS. Podocyte function depends on the integrated structural and signaling roles of a large number of interacting foot process proteins, ranging from nephrin and Neph1 at the slit diaphragm, to scaffolding proteins such as CD2AP and ZO-1, to the actin cytoskeleton and its “bundling” protein, α -actinin-4. In aggregate, heterozygous epistatic modifying gene mutations may not be particularly rare. *NPHS1* heterozygotes alone may have a frequency approaching 1% in many populations, and there may be dozens of genes (e.g., *FAT1*, *FAT2*, *Neph1*, *Neph2*, *P-cadherin*, *CASK*), mutations of which could have epistatic interactions with “classical” homozygous or compound heterozygous mutations in genes previously recognized as associated with SRNS. Clinical phenotypes are being increasingly appreciated for heterozygous mutations in genes originally implicated in classic Mendelian recessive diseases.¹⁴ In well-mixed communities, it is possible that digenic or triallelic combinations of heterozygous mutations of interacting genes are more common than classical “pure” autosomal recessive cases in SRNS, albeit perhaps more subtle in their presentation.

The findings of Hinkes *et al.*³ and Philippe *et al.*⁶ and their colleagues highlight some of the limitations of the purely descriptive, clinicopathologic diagnoses of SRNS that we use today. We still lack a robust molecular diagnostic framework for many cases of SRNS. Integration of patient genotype for multiple podocyte-expressed genes and biopsy gene expression¹⁵ together with novel histologic descriptions based on podocyte number or patterns of expression of specific podocyte proteins may allow us to parse complex genetic forms of SRNS into a limited number of disease-related modules that better reflect physiologically linked intracellular systems of podocyte proteins. The complexity of the genetics may seem daunting, but the opportunity to forge new pathogenetic and diagnostic insights may be worth the effort.

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DISCLOSURES

None.

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See related article, “Nephrin Mutations Can Cause Childhood-Onset Steroid-Resistant Nephrotic Syndrome,” on pages 1871–1878.