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EDITORIAL COMMENTARY

NPHS3: new clues for understanding idiopathic nephrotic syndrome

Bernward G. Hinkes

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Abstract Hereditary forms of childhood nephrotic syndrome (H-CHNS) have long been counted as rare variants of steroid-resistant nephrotic syndrome (SRNS). This concept must be specified by two new findings: First, a study on nephrotic syndrome manifesting in the first year of life documents that H-CHNS are actually the predominant cause of nephrotic syndrome in infants. Second, the recent identification of autosomal recessive nephrotic syndrome type 3 (NPHS3) caused by mutations in the phospholipase *PLCE1* gene has, for the first time, shown steroid responsiveness in H-CHNS. NPHS3 is a severe form of isolated nephrotic syndrome with rapid progression to terminal renal failure. NPHS3 is caused by a developmental rather than structural podocyte dysfunction and is a major cause of diffuse mesangial sclerosis. Therapy response in NPHS3 is documented and could open insights into direct genomic and nongenomic effects of glucocorticoids on podocytes. The findings on NPHS3 support the idea that both clinical course and histology in H-CHNS are subject to genotypic variability and that mutational analysis is the most reliable diagnostic tool. Future studies are needed to determine the clinical implications of NPHS3. Identification of further variants of H-CHNS can be anticipated and may include steroid-responsive hereditary diseases.

B. G. Hinkes (✉)
Klinik für Kinder und Jugendliche,
Friedrich-Alexander-Universität Erlangen-Nürnberg,
Loschgestrasse 15,
91052 Erlangen, Germany
e-mail: bernward_hinkes@hotmail.com

B. G. Hinkes
Department of Pediatrics,
University of Michigan, 8220C MSRB III,
1150 West Medical Center Drive,
Ann Arbor, MI 48109-0646, USA

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Nephrotic syndrome (NS) is the association of gross proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Descriptive classifications for childhood NS (CHNS) are still predominant. According to patients' response to a widely used standard steroid treatment, CHNS is either classified as steroid sensitive or steroid resistant. In the majority of cases, the underlying pathophysiology of CHNS in not understood, and the mode of glucocorticoid action is not yet conceived. Whereas various findings support the idea that steroid-responsive and steroid-resistant forms of CHNS have distinct causes, the question of an overlap of or even a unifying explanation for both categories has been raised [1].

The current hypothesis is that on the one hand side, T-cell-associated processes and the effects of circulating permeability factors underlie a majority of steroid-sensitive cases [2]. In this concept, systemic anti-inflammatory or immunosuppressive effects of glucocorticoids are made responsible for resolving NS symptoms. The less frequent steroid-resistant cases of CHNS are, on the other hand, considered the result of structural glomerular anomalies. They include hereditary forms of childhood NS (H-CHNS), which have been discovered by positional cloning over the last 10 years. Identification of these genes that cause H-CHNS has significantly deepened our understanding of NS in general:

Studies on hereditary forms of NS in children and adults and in animal models have shown that the glomerular podocyte with its filtration barrier of the slit diaphragm between podocyte foot processes is the crucial site where NS evolves. The positional cloning of nephrin [3] and podocin [4] in H-CHNS identified two major, formerly completely unknown, protein components of this glomeru-

lar filter. Both proteins have meanwhile been studied extensively as the cause of NS of the Finnish type (CNF, NPHS1) and of steroid-resistant NS type 1 (SRN1, NPHS2). In addition, mutations in the *WT1* [5] and *LAMB2* genes [6] had been known from syndromic forms of H-CHNS and are now also recognized as rarer causes of isolated CHNS.

A recent study showed that, taken together, mutations in these four genes—*NPHS1*, *NPHS2*, *WT1* and *LAMB2*—explained more than 90% of all cases of isolated congenital NS (manifestation 0–3 months of age at onset) and two thirds of isolated infantile (4–12 months of age at onset) NS in a large cohort of European children [7]. The study emphasized that NS in infants is predominantly a monogenetic disease caused by mutations in these four genes. No response to standard glucocorticoid treatment regimes was observed in patients affected by mutations in any of these four known forms of H-CHNS. The question if mutational analysis prior to any treatment attempt should be obligatory in infants with NS on the basis of these findings was raised.

"Hereditary" had been synonymous with "therapy-resistant" NS in the past, a concept now suddenly questioned by the discovery of a new autosomal recessive form of NS called NPHS3 [8] with responsiveness in some cases.

The first characterization of NPHS3 showed that it manifests as severe isolated NS within the first years of life. Untreated NPHS3 leads to rapid progression to terminal renal failure. The mutated gene *PLCE1* and its gene product phospholipase C epsilon are unanticipated players in the context of glomerular pathophysiology. As an enzyme, phospholipase C epsilon generates second messengers, which regulate various processes affecting cell growth, differentiation, and gene expression. In contrast to podocin (NPHS2) and nephrin (NPHS1), PLCE1 was not enriched directly at the slit diaphragm, but was rather present in the cytoplasm. Whereas the picture of PLCE1 function is still incomplete, first data indicate a functional connection between PLCE1 and diaphragmatic proteins such as podocin and nephrin. Diminished podocin and nephrin expression were observed in the glomeruli of children with *PLCE1* mutations. Furthermore, PLCE1 interaction with the assembly protein IQGAP1, a known player at the slit diaphragm, was shown. The hypothesis that hereditary forms of CHNS are caused by defects of structural components of the podocyte could, in the case of NPHS3, require a modification. Structural anomalies in NPHS3 may not result from a dysfunctional static component of the podocyte but, rather, represent the result of a misled structural development of the cell. The postulated function of PLCE1 would lie in driving and guiding correct structural development of the podocyte. Histologically, fully lacking PLCE1 activity results in developmental arrest of the whole glomerulus, which is known as diffuse mesangial sclerosis (DMS). DMS was observed in all NPHS3 patients with truncating *PLCE1* mutations identified to date. It is the result of the combination of blocked maturation of glomerular capillary loops, equivalent to the differentiation stage at 23 gestational weeks in nonaffected fetuses, with consecutive mesangial proliferation. Interestingly, DMS has also been observed in H-CHNS due to *WT1* and *LAMB2* mutations [5, 6]. The search for a unifying explanation for H-CHNS caused by *NPHS3*, *WT1*, and *LAMB2* mutations could thus be a very rewarding endeavor.

The assumption that histology is not specific for and may be diverse even in one and the same form of H-CHNS is emphasized by another finding on NPHS3. A missense mutation of *PLCE1* resulted in focal segmental glomerulosclerosis (FSGS) rather than DMS. This recalls similar finding in NPHS2 where the majority of mutations present as FSGS, whereas severe *NPHS2* mutations, such as the R138Q founder mutation, may mimic the histology of Finnish type NS, otherwise observed in NPHS1 [7]. One may raise the question if histology in those cases could thus reflect a time point of damage to the developing glomerular filter or a chronology of damage to the glomerulus rather than a characteristic feature of a specific genetic disease. In such a scenario, DMS caused by mutations in *NPHS3* would represent a developmental arrest due to lack of PLCE1 function, whereas FSGS in patients with *NPHS3* missense mutations could result from a more chronic glomerular lesion in the presence of diminished, but remaining, enzymatic activity of PLCE1. This concept of a genotype–phenotype correlation in NPHS3 is also supported by clinical findings. A less severe and slower clinical course with initially conserved glomerular function could be observed in patients with the milder missense mutations of *NPHS3*. Similar genotype–phenotype correlations in H-CHNS have also recently been described for patients with *LAMB2* or *NPHS2* mutations [9, 10]. Based on these results and given the high likelihood of detecting mutations in CHNS among infants, genetic testing would be the more reliable diagnostic tool than histology for early onset NS.

A most unexpected finding about NPHS3 was the sustained complete response of a 1-year-old girl from a consanguineous family to standard steroid treatment. She was affected by a homozygous truncating *NPHS3* mutation, which both her healthy parents carried heterozygously. This observation is remarkable, as it documents the first example of complete therapy response in a patient with a severe form of hereditary NS. The fact that the successfully treated patient was indeed affected by a life-threatening truncating *PLCE1* mutation is emphasized by the clinical course of her aunt, who had carried the identical homozygous stop codon mutation. This aunt presented at 8 months of age with NS

and DMS, had not received any treatment, and had died of the disease at 11 months of age. This is particularly important, as no biopsy has ever been obtained from the cured girl due to her rapid complete remission. This histology of the responsive patient would be an important piece in the puzzle, as it would open direct insights into the effects of glucocorticoids action in NPHS3. Therefore, one can only speculate that the treatment response reflects the gain of a regular glomerular function in this child. As regular glomerular function is incommensurate with the developmental arrest documented in the aunt with the identical mutation, the arrest should have been bypassed in the cured girl. A very reasonable explanation for this effect would be an alternative stimulus substituting for PLCE1 itself, or for unknown downstream effects of PLCE1. How could this have happened? It is known that all subsets of glomerular cells, including podocytes, carry the glucocorticoid receptor in the nucleus and the cytoplasm [11]. With regard to NS, direct genomic and nongenomic effects of corticoids on the podocyte have received less attention than anti-inflammatory or immunosuppressive mechanism. In the context of NPHS3, those direct effects could now play a key role. The up-regulation of a substitutional phospholipase enzyme by glucocorticoids would be a rather simple but very logic explanation. In addition, downstream effects of PLCE1 may have been activated directly by glucocorticoids as well. These could include the following known glucocorticoid effects on the podocyte: Glucocorticoids stabilize the actin cytoskeleton [12] and have been shown to actively induce actin assembly [13]. Through their impact on actin polymerization, glucocorticoids protect podocytes and enhance podocyte recovery after damage [14]. Known direct genomic effect of glucocorticoids on podocytes further include up-regulation of glomeruloprotective proteins [15], up-regulation of nephrin, suppression of harmful cytokine production [16–18], and prevention of podocyte apoptosis [19]. Finally, glucocorticoids may positively influence podocyte proteoglycan synthesis, which is essential for regular podocyte morphology and regular anionic charge of the glomerular filter [20].

In the end, the clinician will ask for the clinical consequence of these finding on NPHS3. In particular, the question could be whether all infants with NS should be treated with glucocorticoids now that NPHS3 is known. Our current knowledge is that, despite the discovery of NPHS3, the majority of children with NS manifesting in the first year of life still did not respond to steroids. A recent study on children with isolated DMS found truncating *PLCE1* mutations in more than 30% of all those affected [21]. As NPHS3 could therefore be a major cause of DMS, a treatment attempt in the subgroup of children with DMS may be justified. But DMS is rare, and in response, the clinical researcher may first have to ask how many children with unrecognized mutations in *NPHS3* exist, how these children have been treated, and what the outcome of such treatment was. To determine the role of NPHS3 among all children with NS however, the challenging subject of future studies will be to also analyze patients presenting with FSGS or simply with steroid-sensitive NS for mutations in *NPHS3*. The result of such studies is hard to predict and may be full of surprises, as can be seen from examples such as NPHS2. NPHS2 was discovered in only six kindred of North African descent but is now known as the most common hereditary form of CHNS, causing at least in one out of five cases of SRNS in children [4, 22, 23].

In summary, the lesson already learned from NPHS3 and other forms of H-CHNS is that NS in general is more complex than current descriptive classifications according to therapy response, age at onset, or histology. In analogy to other kidney diseases such as nephronophthisis, a vast number of genetic forms of NS may be waiting for discovery and may further deepen our general understanding of the kidney and its dysfunctions. In particular, the search for steroid-responsive forms of H-CHNS has become more promising than ever before through the discovery of NPHS3.

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