The Heart of Children with Steroid-Resistant Nephrotic Syndrome: Is It All Podocin?

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Mutations in the gene NPHS2 encoding podocin are responsible for a recessive form of steroid-resistant nephrotic syndrome (SRNS). The common phenotype is of massive proteinuria in early childhood that tends to progress to end-stage renal failure. Extrarenal manifestations have not been described. Twenty-two children with SRNS from six unrelated Arab families were found to be homozygous for the R138X mutation in NPHS2. Eighteen patients underwent cardiac evaluation at diagnosis of SRNS while they had normal BP and preserved renal function. Cardiac anomalies were detected in 16 (89%) children: Left ventricular hypertrophy in eight, pulmonary stenosis in six, discrete subaortic stenosis in two, and Ebstein anomaly and ventricular septal defect in one each. The remaining four affected individuals were assessed only once they had end-stage renal failure. They had severe left ventricular hypertrophy and experienced repeated episodes of heart failure. Two control groups were equally evaluated. The first consisted of 37 siblings without nephrotic syndrome, of whom only one carrier had a cardiac defect (P < 0.001). None of the second group, which included 22 children with persistent nephrotic syndrome as a result of other causes, had a cardiac anomaly (P < 0.001). Cardiac disorders in homozygotes for mutations in NPHS2 cannot be attributed to an association by chance or to a state of persistent nephrotic syndrome. Because human podocin mRNA is expressed in fetal heart, it is speculated that it may have a role in normal cardiac development. Cardiac evaluation is recommended at the time of diagnosis of SRNS due to mutations in podocin.


Nephrotic syndrome (NS) is defined as the combination of massive proteinuria, hypoalbuminemia, and edema. In recent years, the molecular bases of several conditions that lead to steroid-resistant nephrotic syndrome (SRNS) have been identified (1,2). The common denominator shared by these clinical entities is that they all result from a structural defect in the glomerular barrier, thus explaining their unresponsiveness to immunosuppressive agents (3). There is significant overlap between these disorders with respect to the age of onset, the severity of NS, the histologic findings, and the clinical outcome. The congenital NS of the Finnish type (CNF) is caused by mutations in the NPHS1 gene encoding nephrin, which is expressed in the slit diaphragm joining the podocyte foot processes (4). Mutations in ACTN4, encoding α-actinin-4, cause a dominant form of focal segmental glomerulosclerosis (FSGS) (5). A recessive form of SRNS was found to result from mutations in NPHS2 encoding a protein named podocin as a result of its specific expression in podocytes (6).

We previously reported that a founder mutation in podocin (R138X) is a prevalent cause of SRNS in Arab children (7). Subsequently, it was noted that a number of these patients have a co-existing cardiac disorder. Only a few case reports described an association between SRNS and cardiac defects (8,9). The aim of this study was to review systematically the cardiac status of these SRNS patients at the time of diagnosis and during the course of the disease. This was particularly intriguing because genetic glomerular barrier disorders have been considered to be kidney specific.

Materials and Methods
Twenty-two children (12 male) with SRNS from six consanguineous kindreds of Arab descent have been diagnosed and treated in our center. These families are unrelated to each other. Genetic analysis showed that all of the patients were homozygous for the nonsense R138X mutation in NPHS2, encoding podocin. Four children were referred to our center once they already had ESRD, and the diagnosis of SRNS was based on clinical characteristics and confirmed by genetic studies. These patients lacked comprehensive cardiac assessment at the time of diagnosis of SRNS but underwent complete evaluation once renal replacement therapy was initiated.

Cardiac evaluation that was performed within 0.9 ± 1.5 mo from the time of diagnosis in the remaining 18 children included physical examination, chest radiogram, electrocardiogram, and echocardiography. Echocardiogram was performed by senior pediatric cardiologists using a Sonos 2000 machine. The study included detailed M-mode measurements, two-dimensional echocardiography, and color Doppler interrogation. Left ventricular hypertrophy (LVH) was defined as left ventricular septum or posterior wall thickness that was greater than the 90th
percentile for body surface area (10). All patients were subsequently followed on a regular basis by the pediatric cardiology team.

Chronic kidney disease (CKD) was defined as calculated GFR < 80 ml/min per 1.73 m². Two control groups were selected. The first one consisted of 37 siblings (19 male) without NS, and the second group included 22 children (13 male) with persistent NS as a result of various causes other than mutations in podocin. NS in the second control group was due to isolated diffuse mesangial sclerosis in five children, membranoproliferative glomerulonephritis in four, steroid-dependent NS in four, FSGS in three, CNF and Alport syndrome in two each, and familial Mediterranean fever (FMF)-associated amyloidosis and Henoch-Schönlein nephritis in one each. Children of both control groups underwent complete cardiac evaluation. Comparison of proportions of cardiac anomalies between the various groups was performed using t test. This study was approved by the Ethics Committee of the Shaare Zedek Medical Center.

Results

SRNS was diagnosed at the mean age of 2.3 ± 3.1 yr with neonatal presentation in three infants. Sixteen children from this cohort reached ESRD during their first decade of life, and eight underwent kidney transplantation.

Cardiac anomalies were detected in 16 (89%) of 18 children at the time of diagnosis of SRNS, while they all had normal BP and preserved kidney function (Table 1). The most common finding was of LVH, detected in eight children, followed by pulmonary stenosis (valvular, supravalvular, or peripheral pulmonary artery stenosis) in six children. Discrete subaortic stenosis was detected in two children, whereas Ebstein anomaly of the tricuspid valve and ventricular septal defect were detected in one patient each. Only two children had a normal cardiac examination at diagnosis of SRNS.

| Table 1. Cardiac anomalies in children with steroid-resistant nephrotic syndrome as a result of mutations in podocina |
|-----------------|-----------------|
| Patient | Cardiac Defect |
| AS-1 | LVH |
| AS-2 | LVH |
| AA | LVH |
| S-1 | VSD, LVH |
| S-2 | Discrete subaortic stenosis |
| S-3 | Discrete subaortic stenosis |
| AR-1 | Pulmonary valve stenosis, LVH |
| AR-2 | LVH |
| AR-3 | LVH |
| AR-4 | LVH |
| AR-5 | Supravalvular pulmonary stenosis |
| AR-6 | Supravalvular pulmonary stenosis |
| M-1 | Ebstein anomaly |
| M-2 | Peripheral pulmonary artery stenosis, biventricular hypertrophy |
| G-1 | Pulmonary valve stenosis |
| G-2 | Pulmonary valve stenosis |

*aLVH, left ventricular hypertrophy, VSD, ventricular septal defect.

Factors such as anemia and hypocalcemia, which potentially could have an impact on the cardiac abnormalities, were evaluated at the time of diagnosis of SRNS and were excluded. The mean serum hemoglobin level was 11.1 ± 1.1 g/dl, and the mean serum ionized calcium was 1.23 ± 0.048. The mean plasma albumin was 1.8 ± 0.4 g/dl.

In two instances, the diagnosis of cardiac defect preceded that of NS. Patient G-1 (Table 1) was evaluated for generalized weakness at the age of 11 yr. Echocardiography revealed severe pulmonary valve stenosis with a gradient of 78 mmHg, which was managed successfully by balloon valvuloplasty. One year later, his 2.5-yr-old brother (G-2) was diagnosed with SRNS, and screening of all siblings demonstrated that G-1 had asymptomatic NS with a homozygous R138X genotype. Patient AR-4 was referred to a pediatric cardiologist for evaluation of shortness of breath and edema. Echocardiography showed concentric hypertrophic cardiomyopathy, and a complete work-up led to the diagnosis of SRNS.

Intrafamilial variability in cardiac defects was demonstrated in several kindreds. M-1 and M-2 are second cousins (Figure 1): One had Ebstein anomaly of the tricuspid valve, and the other had peripheral pulmonary artery stenosis with biventricular hypertrophy. In family AR (Figure 1), four children received a diagnosis of LVH, and three received a diagnosis of pulmonary stenosis. In family S, one child had hypertrophic cardiomyopathy, and the other two had discrete subaortic stenosis. In one child (S-2), it mandated two cardiac surgeries in which a membrane was resected. His most recent echocardiography showed no residual stenosis.

Analysis of serial echocardiograms among our study patients demonstrated mild to severe LVH already at the time of diagnosis of SRNS, before the development of CKD, which was documented much later at the mean age of 5.1 ± 2.5 yr. Further augmentation in LVH was noted with age, comitant with the development of significant hypertension and gradual decline in renal function. LVH peaked with onset of ESRD, leading to reduction in left ventricular systolic function and resulting in repeated episodes of congestive heart failure. Six of eight patients with LVH were treated with angiotensin-converting enzyme inhibitors as antiproteinuric agents. This therapeutic modality had absolutely no effect on the course of LVH.

Four children who received a diagnosis of LVH, early in life and before hypertension or renal failure evolved, underwent a successful kidney transplantation at the mean age of 6.5 ± 1.1 yr. It is interesting that complete resolution of their LVH occurred within a period of 2 to 4 yr at the mean age of 9.2 ± 1.2 yr.

Cardiac evaluation of all four children who were diagnosed with SRNS as a result of mutations in podocin when they already had ESRD demonstrated severe LVH. These patients experienced repeated bouts of congestive heart failure.

Thirty-seven siblings without NS (first control group) underwent cardiac evaluation at the mean age of 7.4 ± 2.9 yr: 16 had normal genotype, and 21 were heterozygotes for the R138X mutation in podocin. None of the individuals with normal genotype had a cardiac disorder, and only one carrier was found to have cardiac anomaly, which consisted of a medium-
sized atrial septal defect and mild pulmonary valve stenosis with a gradient of 27 mmHg.

All 22 patients who were aged 4.6 ± 3.9 yr and had severe persistent NS as a result of various causes other than mutations in podocin (second control group) had normal cardiac anatomy and function. The incidence of cardiac anomalies among patients with SRNS as a result of mutations in podocin was significantly higher than that of both control groups ($P < 0.001$ versus healthy siblings and $P < 0.001$ versus children with persistent NS as a result of other causes).

**Discussion**

We detected congenital cardiac anomalies in 16 of 18 children at the time of diagnosis of SRNS as a result of the homozygous R138X mutation in podocin. The identification of a nonsense mutation, resulting in truncation of an integral structural component of the glomerular barrier, explains the phenotype of massive proteinuria and SRNS. The leading cardiac defect was LVH followed by pulmonary stenosis and less frequently discrete subaortic stenosis, Ebstein anomaly of the tricuspid valve, and ventricular septal defect. The remarkably increased incidence of specific cardiac defects in children with SRNS described here exceeds by far the reported incidence of these anomalies in the general population (11) and points against a random association. For instance, pulmonary stenosis was detected in 33.3% of patients with SRNS compared with only 0.08 to 0.15% in the general population (12).

The association between familial SRNS and congenital cardiac malformations may be attributed to three distinct mechanisms: Mutated podocin, mutations in a gene that is closely linked to podocin, or homozygous mutations in an unlinked gene as expected in a highly consanguineous population such as that reported here. If cardiac anomalies and SRNS were two separate traits, then one would expect to find a similar incidence of cardiac defects among siblings who are not homozygous for a mutation in podocin. In fact, none of the unaffected siblings with normal genotype had a cardiac disorder, and only one carrier of the mutation had a heart anomaly. This proves unequivocally that cardiac anomalies and SRNS as a result of mutations in podocin do not represent an association by chance but rather are co-inherited. Whether the cardiac disorder that was detected in a carrier represents the effect of one podocin-mutated allele and a second somatic mutation in podocin or a different gene remains to be determined (13).

Nephrotic syndrome, unaccompanied by hypertension or renal failure, has not been shown to engender cardiac anomalies. This notion was substantiated by our finding that none of the studied 22 children with severe persistent NS as a result of various causes other than mutations in podocin had a cardiac anomaly. Therefore, massive proteinuria per se cannot account for the increased incidence of heart defects.

The first association between familial NS and cardiac involvement was reported over 40 yr ago (8). Four sisters, born to healthy parents, had SRNS that progressed to ESRD and were found to have cardiac murmurs. Further evaluation that was performed in two girls revealed infundibular pulmonary stenosis. Because this report preceded the definition of the molec-

**Figure 1.** Pedigrees of six families with steroid-resistant nephrotic syndrome (SRNS) as a result of the homozygous R138X mutation in NPHS2 encoding podocin, demonstrating co-inheritance with cardiac defects. Full symbol, homozygote; half-full symbol, heterozygote; empty symbol, healthy; ◊, cardiac anomaly detected at diagnosis of SRNS; ⊠, cardiac anomaly detected when patient had ESRD.
ular bases of the various recessive forms of SRNS, the exact cause of their renal disease remained unknown. Grech et al. (9) described four children who were from Malta and had CNF and cardiac malformations: Three had valvular pulmonary stenosis, and one had discrete subaortic stenosis. The diagnosis of CNF was based on histologic findings of kidney biopsies. It is unclear whether these four children were enrolled in a later study of the same population which demonstrated that there is a founder mutation in NPHS1 (R1160X) in children with CNF, with marked renal phenotypic variability (14). The authors concluded that the likelihood that two such rare conditions (CNF and these particular cardiac malformations) would be due to chance alone is low. A larger survey detected cardiac disorders in 12 of 42 children with CNF, which included pulmonary stenosis, cardiac hypertrophy, and atrial septal defect (15). The distribution of co-existing cardiac anomalies was even in all genotypes tested, whether they were homozygotes or compound heterozygotes. Cardiac hypertrophy was attributed by these researchers to hemodynamic changes resulting from hypoproteinemia, as marked improvement was noted after bilateral nephrectomy. A child who was from Turkey and had SRNS as a result of three different homozygous mutations in podocin and cardiac malformation, consisting of aortic valve stenosis with regurgitation, was presented recently (16). This case, which occurred in a child from a different population from ours and involved other mutations in podocin, supports our observation of the co-inheritance of these two traits.

The heart defects that were detected in our patients are similar to those reported in other children with familial SRNS and CNF, including primarily cardiac hypertrophy, pulmonary stenosis, and discrete subaortic stenosis (8,9,15). That a number of cardiac anomalies are shared by patients with CNF favors the mechanism of direct effect of nephrin and/or podocin instead of an indirect effect of a closely linked mutated gene.

Several lines of evidence support a possible role for these genes in normal heart development. The expression of nephrin in nonrenal tissues is not completely known: Northern hybridization did not reveal nephrin-RNA in fetal placenta, liver, brain, lung, muscle, or pancreas, but it was not performed in human fetal heart (4). In mouse embryos, nephrin was shown to be expressed in the kidney, the central nervous system, and the pancreas (17,18). There was no expression of murine nephrin in fetal heart.

Human podocin mRNA was found to be strongly expressed both in fetal and adult kidney. It is interesting that there was also a positive signal in human fetal heart but not in adult heart (6). The renal phenotype of podocin-deficient mice has been studied extensively, but no information has been provided concerning their cardiac status (19). An association has been described recently between heart disease and FSGS but not other forms of primary NS, with no significant difference in BP or hematocrit levels (20). Cardiac involvement included LVH and congestive heart failure. These patients had not been genotyped for mutations in podocin, and no data were available concerning their cardiac status at the time of diagnosis of FSGS.

Nephrin, encoded by NPHS1, is an integral membrane protein of the Ig superfamily and is a critical structural component of the slit diaphragm. The cytoplasmic tail of nephrin is tyrosine phosphorylated and recruits signaling intermediates that bind to the phosphorylated tyrosine residues, rendering it a signal transduction protein. Podocin, in turn, is a member of the stomatin protein family with a predicted hairpin-like structure localized to the insertion site of the slit diaphragm of podocytes. Podocin has been shown to be lipid-raft associated at the filtration slit (21). Lipid rafts are specialized microdomains of the plasma membrane with a unique lipid composition that are highly concentrated in signal transduction molecules. Podocin serves to recruit nephrin into lipid-raft microdomains, which is required for the proper initiation and augmentation of nephrin signaling (22). Studies of the effect of the R138X mutant in podocin, which is responsible for SRNS in our patients, showed that podocin may still interact with nephrin and get to the plasma membrane, but they are not targeted to the rafts. Therefore, this mutant cannot augment nephrin signal transduction.

Several genes have been identified as being responsible for various congenital heart diseases (23). It has been shown that mutations of most congenital heart diseases genes can engender more than one cardiac phenotype. LVH and pulmonary stenosis were the two most prevalent cardiac disorders detected in our patients with SRNS.

We speculate that the association between SRNS as a result of mutations in podocin and cardiac anomalies points to a causal role of podocin in normal cardiac development. The podocin transcript is expressed in fetal heart, and its product may interact with other peptides and affect the overall signal transduction. Mutated podocin may lead to abnormal signaling, resulting in congenital heart defects. The possibility that SRNS and cardiac defects are co-inherited but result from two closely linked mutated genes cannot be excluded on the basis of current data.

That all four patients with LVH recovered normal heart anatomy and function after successful kidney transplantation may reflect the compensatory effect of other genes later on in life, as podocin is not expressed in human adult heart. A similar pattern of complete resolution of hypertrophic cardiomyopathy was described previously in children with the idiopathic (or familial) form and in a child with Noonan syndrome (24). Because the natural history of podocin-associated SRNS is of ESRD during the first decade of life, it precludes the systematic study of LVH during this period independent of hypertension or CKD. Humoral factors that are associated with chronic renal failure and that improved after renal transplantation as well as better control of hypertension may also play a role in regression of the LVH (25).

Because heart anomalies are prevalent among children with SRNS as a result of mutations in podocin, complete cardiac evaluation is recommended at the time of diagnosis to enable proper follow-up and early intervention when indicated. Cardiac defects in these patients are not a contraindication to renal transplantation and have no bearing on the timing of transplantation. More studies are needed to document an increased incidence of cardiac anomalies among children with SRNS as a result of different mutations in NPHS2. Further research is mandatory to define precisely the mechanism underlying the
association between SRNS as a result of mutations in podocin and heart defects.

References

Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/