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Low prevalence of *NPHS2* mutations in African American children with steroid-resistant nephrotic syndrome

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Abstract

In African American (AA) children, focal segmental glomerulosclerosis (FSGS) is the leading cause of nephrotic syndrome (NS). It has been shown that AA children suffer from FSGS and steroid-resistant nephrotic syndrome (SRNS) at a higher frequency and with a more severe renal outcome in comparison with Caucasian children. Previous mutation analysis of large cohorts revealed that a high percentage of childhood SRNS is monogenic and that mutations in podocin (*NPHS2*) and Wilms' tumor gene 1 (*WT1*) account for approximately 30% of SRNS in children. To test whether AA children with SRNS have a similar or a higher mutation rate, we performed mutation analysis of *NPHS2* and *WT1* in a cohort of AA children with SRNS. Direct sequencing was carried out for all exons of *NPHS2* and for exons 8 and 9 of *WT1*. We ascertained 18 children

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of AA descent in whom renal biopsy findings showed FSGS in 13 patients (72%) and minimal-change disease in five patients (28%). In both *NPHS2* and *WT1*, no disease-causing mutations were detected. Our data strongly suggest that in AA children with SRNS, the frequency of *NPHS2* mutations is much lower than in large cohorts of pediatric SRNS patients in the general population. Knowledge of mutation rate of *NPHS2* in different populations of SRNS patients facilitates the physician in planning a suitable genetic screening strategy for patients.

Keywords

NPHS2; African American; Mutations; WT1; Nephrotic syndrome

Introduction

Nephrotic syndrome (NS) in children and adolescent is traditionally separated into two categories on the basis of response to standard steroid treatment: steroid-sensitive nephrotic syndrome (SSNS) versus steroid-resistant nephrotic syndrome (SRNS) [1]. Early reports estimated SRNS to occur in approximately 10% of all children with sporadic NS [1, 2], whereas a recent study found 27% of children with NS to be eventually resistant to steroids [3]. Renal histology in children with SRNS reveals in about 75% of children the findings of focal segmental glomerulosclerosis (FSGS) and in another 20% minimal-change disease (MCD) [1, 2]. Previous reports have shown that FSGS is the main cause of idiopathic NS in African American (AA) children [4, 5]. It has also been reported that AA children, compared with Caucasian children, have a higher prevalence of SRNS, present with NS at an older age with a more rapid deterioration towards end-stage renal failure (ESRF), but have a lower rate of FSGS recurrence following kidney transplantation [3–7].

In recent years, mutations of several recessive and dominant genes were found to be sufficient as the only cause of SRNS in patients with familial or sporadic SRNS. Mutations causing recessive SRNS were found in nephrin (*NPHS1*) [8], podocin (*NPHS2*) [9], laminin-beta-2 (*LAMB2*) [10, 11], phospholipase C epsilon 1 (*PLCE1*) [12], and CD2-associated protein (*CD2AP*) [13] genes. SRNS in children is also associated with dominant mutations in the Wilms' tumor 1 gene (*WT1*) [14]. In adults, dominant mutations in alpha-actinin 4 (*ACTN4*) [15], transient receptor potential cation channel 6 (*TRPC6*) [16], and *CD2AP* [17] genes were found to cause hereditary forms of FSGS. Identification of these genes significantly increased our knowledge of glomerular biology with a strong emphasis on the role of the podocyte and the glomerular slit diaphragm in the mechanism of glomerular filtration [18].

Since the identification of *NPHS2*, we and others have demonstrated that recessive mutations of *NPHS2* account for 10.5–28% of idiopathic pediatric SRNS in large cohorts of pediatric SRNS patients, mostly from Caucasian and Asian descent [19–21]. Mutation analysis of children with SRNS from different ethnic backgrounds revealed variable results. In Israeli Arab children with familial and sporadic SRNS, an *NPHS2* mutation rate of 55% was found, which is higher than reported in worldwide cohorts [22]. Two separate Turkish cohorts of SRNS patients found an *NPHS2* mutation rate of 27.4% [23] and 13.3% [24], and

a Chinese study found a mutation rate of 15.9%, similar to the findings in worldwide cohorts [25]. On the other hand, a lower 4% prevalence of *NPHS2* mutation rate was found in a different Chinese cohort [26] and in one out of 13 Japanese children with congenital NS [27]. No *NPHS2* mutations were detected in a large cohort of South Korean children with sporadic and familial SRNS [28], pediatric SRNS patients from Japanese descent [29, 30], and Israeli Jewish children with SRNS and biopsy-proven FSGS [22]. Several studies have also screened SRNS patients for mutations in *WT1*. The rate of disease-causing mutations was similar (5–9%) for both large cohorts of pediatric SRNS patients mostly from Caucasian and Asian descent and for patients from South Korea and Italy [14, 28, 31].

Interestingly, despite the distinctive clinical course of FSGS in AA, there is only scant data regarding a possible genetic cause for FSGS or SRNS in this ethnic group. Direct sequencing of *NPHS2* in 96 AA adult patients with nondiabetic ESRF, in comparison with AA healthy individuals as controls, did not reveal homozygous or compound heterozygous mutations in *NPHS2* [32]. It was demonstrated that rare variants of *NPHS2* and particularly IVS3+9insA may contribute to the development of ESRD in these patients. McKenzie et al. investigated the sequence variants of the *NPHS2* gene in sporadic late-onset FSGS without finding any homozygous or compound heterozygous mutations in 247 AA patients [33]. A strong association was suggested between a common haplotype of noncoding single nucleotide polymorphisms (SNPs), carried by 20% of AA, and a reduced risk for FSGS [33].

A possible role for *WT1* and the neighboring gene Wilms'-tumor-associated protein (*WIT1*) in the pathogenesis of FSGS in AA was suggested by Orloff et al., demonstrating that several SNPs in *WT1* and *WIT1* are significantly associated with the occurrence of idiopathic FSGS and HIV-1-associated FSGS in this ethnic group [34]. A genomewide linkage scan for a large AA family with dominantly inherited nephropathy mapped a locus on chromosome 9q31–q32 [35]. The 13-year-old male proband of this family had renal histology findings of FSGS.

Given the possible role of *NPHS2* and *WT1* in the pathogenesis of NS in AA and the unique clinical course seen in children from this ethnic group, we performed mutation analysis of *NPHS2* and *WT1* in a cohort of AA children with SRNS. We tested whether the *NPHS2* and *WT1* mutation rate for this group is similar or higher to that in previously described cohorts of other ethnic groups. We hypothesized that the distinctive clinical course seen in AA children with FSGS might be related to a higher percentage and more severe mutations in *NPHS2* and *WT1*.

Methods

Patients and data recruitment

DNA samples and clinical data from 18 AA children with nonsyndromic SRNS were ascertained between 2004 and 2007. Approval of the study was granted by the Institutional Review Board (IRB) of the University of Michigan, Ann Arbor. Patient recruitment following informed consent has been described previously [20]. Diagnosis of SRNS was established by a pediatric nephrology specialist at different pediatric nephrology centers, according to published criteria. Exclusion criteria were: (a) known secondary cause for NS

(e.g. Hodgkin's disease); (b) age >16 years; (c) absence of extrarenal manifestations. For clinical evaluations, we used a standard questionnaire (www.renalgenes.org). Characteristic features defining the clinical diagnosis were age of onset, response to steroid or other immunosuppressive therapy, histological features of kidney biopsy, extrarenal manifestation, interval time of progression toward ESRF, and recurrence of disease after kidney transplantation. Standard steroid treatment and responses to steroid therapy were defined according to the International Study of Kidney Disease in Children and the Arbeitsgemeinschaft fur Pädiatrische Nephrologie guidelines [1, 36]. Nephrotic-range proteinuria was defined as >40 mg/m^2 per hour. Standard steroid therapy was defined as 60 mg/m^2 per day prednisone administered orally in three divided doses for 6 weeks. Primary resistance to steroid treatment was defined as the absence of remission to less than a trace of proteinuria on dipstick analysis or <4 mg/m^2 per hour within the initial 6 weeks of standard steroid therapy.

Mutation analysis of *NPHS2* and *WT1* by direct sequencing

Blood samples of patients were acquired and used for DNA extraction. Genomic DNA was isolated from blood samples using the Puregene® DNA purification kit (Gentra, Minneapolis, MN, USA) following the manufacturer's guidelines. Mutation analysis was performed by exon-flanking polymerase chain reaction (PCR) with consecutive direct sequencing of all eight exons of *NPHS2* and exons 8 and 9 of *WT1*, as described previously [19, 37]. *WT1* analysis was limited to exons 8 and 9, as mutations of this gene accounting for isolated SRNS have only been reported in these two exons [37]. Exon primer sequences are available from the authors upon request. For sequence analysis, the software SEQUENCHER™ (Gene Code Corp, Ann Arbor, MI, USA) was used. For detected mutations and other sequence variants, sequencing of both strands was performed.

Results

Clinical characteristics

Overall, we performed mutation analysis of all eight exons of *NPHS2* and exons 8 and 9 of *WT1* in 18 AA children with SRNS. A summary of clinical data is shown in Table 1. The median age of presentation was 7.2 years. The median age of presentation was 2.2 years older in this group compared with the age found in our previous cohort of SRNS patients mostly from European descent [38]. None of the patients had a positive family history of kidney disease. One patient (A589) is of consanguineous marriage.

Three of the seven patients who progressed to ESRF received a kidney transplant. Of these three patients, two suffered a recurrence of FSGS. One patient (A1649) had a disease course with initial complete remission on tacrolimus therapy for 3 years with later resistance to tacrolimus and progression towards ESRF.

Renal biopsy

A renal biopsy was performed for all patients. Histology diagnosis is shown in Table 1. In 13 patients (72%), the biopsy revealed FSGS. None of these patients had biopsy features of collapsing glomerulopathy. Five patients (28%) had the histological pattern of MCD.

Mutation analysis of *NPHS2*

A summary of results from the mutation analysis of *NPHS2* is given in Table 2. No homozygous or compound heterozygous mutations were found in any of the patients. We detected known innocuous SNPs, which are summarized in Supplementary Table 1. The R229Q amino acid substitution, a sequence variant of unknown significance [39], was not found in any of the patients.

Mutation analysis of *WT1*

A summary of *WT1* mutation analysis is given in Table 2. Known innocuous SNPs are summarized in Supplementary Table 1. We did not detect any disease-causing mutation in *WT1*.

Discussion

Here we present for the first time a mutation analysis of *NPHS2* and *WT1* in a cohort of 18 AA patients with pediatric nonsyndromic SRNS. No homozygous or compound heterozygous mutations were detected in *NPHS2*. Thus, none of the patients had mutations in this gene, which could explain a recessive SRNS. No patients were found to have a disease-causing mutation in *WT1*. The median age of presentation was 7.2 years, which is 2.2 years higher in comparison with our previous worldwide cohort of pediatric SRNS patients [38]. Sorof et al. previously reported a higher age of presentation for 24 AA children with FSGS compared with Caucasian children with FSGS [6].

NPHS2 mutations in children with SRNS bear clinical importance, as children carrying homozygous or compound heterozygous mutations in this gene are distinguished from those without by primary resistance to steroid treatment and by rapid deterioration toward ESRD [20]. On the other hand, patients with FSGS due to recessive mutations in *NPHS2* have a lower rate of FSGS recurrence after kidney transplantation [20, 21]. On the basis of these findings, recommendations were made to screen for *NPHS2* mutations in children with SRNS, as this might enable the physician to better predict disease course and eliminate prolonged unnecessary immunosuppressive therapy.

The lack of *NPHS2* mutations in this cohort of AA children with SRNS is in contrast with previous studies indicating a rate of 10.5–28% mutations in large cohorts of pediatric SRNS [19–21]. However, our findings add to the growing evidence that ethnic difference exists in the rate of *NPHS2* mutations, as not all ethnic groups share the same frequency of mutations in this gene [14, 19–31]. These differences emphasize the need for further *NPHS2* mutation analysis of other ethnic groups in which the frequency of *NPHS2* mutations is still unknown. A better knowledge of mutation rates in different populations of patients might help the physician to plan a suitable genetic screening strategy for patients with SRNS.

The suggestion made by Orloff et al. regarding a possible role for *WT1* and the neighboring gene *WIT1* in AA patients with FSGS was only partially addressed in this study, as only exons 8 and 9 of *WT1* were screened without finding any disease-causing mutation [34]. Future mutation analysis of both genes might shed more light on their possible frequency as the cause of SRNS in AA.

The absence of *NPHS2* and *WT1* mutations in AA patients report here, albeit not in a large cohort, indicates that we cannot attribute the distinctive clinical course previously described in this ethnic group to a higher frequency of mutations in the above-mentioned genes [3–7]. This finding raises the possibility that different and yet unidentified genes are involved in the pathogenesis of SRNS in AA children. Future studies using a total genome search might reveal new gene loci for AA SRNS patients, thereby enabling identification of genes responsible for the development of NS. Identification of such genes will further contribute to our growing knowledge of glomerular biology and might help in finding future therapeutic strategies for genetic as well as nongenetic glomerular diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical data of 18 African American children with steroid-resistant nephrotic syndrome

Clinical parameters	Value
Age onset in years; median (range)	7.2 (0–14.5)
Gender (male; female)	10; 8
Family history of kidney disease (%)	0/18
Renal biopsy (%)	18/18 (100%)
FSGS (%)	13/18 (72.2%)
MCD (%)	5/18 (27.8%)
ESRD (%)	6/18 (33.3%)
Renal transplantation (% of ESRD patients)	2/6 (33.3%)
Recurrence after renal transplantation (%)	2/2 (100%)
Consanguinity (%)	1/18 (5.6%)

ESRD end-stage renal disease, *FSGS* focal segmental glomerulosclerosis, *MCD* minimal-change disease

Table 2

Mutation analysis of *NPHS2* and *WT1* in 18 African American patients with steroid-resistant nephrotic syndrome from 18 different families

Family	Gender	Mutation in *NPHS2*	Mutation in *WT1*	Age of onset (years)	Initial presentation	Response to 2nd line immunosuppression	ESRD (years after onset)	Renal biopsy	Transplantation
A215	M	no	no	13.0	edema	–	4.2	FSGS	no
A589[a]	M	no	no	4.0	edema	–	8.1	FSGS	no
A660	F	no	no	2.8	edema, HTN	–	no	FSGS	no
A968	M	no	no	9.8	incidental	CSA–nd	no	FSGS	no
A1118	F	no	no	4.9	edema	CP–nr	no	MCD (×2)	no
A1194	M	no	no	5.7	incidental	CSA–nr	5.5	FSGS (×2)	no
A1458	M	no	no	4.1	edema	Chl–nr	no	MCD	no
A1478	F	no	no	14.5	edema, RF	CSA–pr	no	FSGS	no
A1483	F	no	no	14.1	edema	CSA–nr	no	FSGS	no
A1500	F	no	no	1.9	edema, HTN	CP, CSA–nr	no	MCD (×2)	no
A1537[b]	M	no	no	0.0	edema	–	5.8	MCD	yes–no recurrence
A1606	F	no	no	12.8	edema, HTN	–	13.4	FSGS (×2)	yes–with recurrence
A1619	M	no	no	11.8	incidental	–	no	FSGS	no
A1647	M	no	no	10.4	edema	Tac–pr (2.5 years)	14.9	MCD–1st FSGS–2nd	yes–with recurrence
A1649	M	no	no	3.9	edema	Tac–cr (for 4 years) then nr	10.2	MCD–1st FSGS–2nd	no
A1657	F	no	no	8.1	edema	–	no	FSGS	no
A1949	F	no	no	6.4	incidental	CSA–nr	no	FSGS	no
A1964	M	no	no	8.2	edema	CSA–pr	no	MCD (×2)	no

M male, *F* female, *Chl* chlorambucil, *CP* cyclophosphamide, *cr* complete remission, *CSA* cyclosporine A, *ESRD* end-stage renal disease, *FSGS* focal segmental glomerulosclerosis, *HTN* hypertension, *MCD* minimal-change disease, *nd* no data, *nr* no response, *pr* partial response, *RF* renal failure, *Tac* tacrolimus, *x2* same diagnosis in two biopsies

[a] Consanguineous family,

[b] found to have a single heterozygous mutation in *NPHS1*