RESEARCH BRIEF

NPHS2 Mutations in Indian Children with Sporadic Early Steroid Resistant Nephrotic Syndrome

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Received: February 23, 2011;
Initial review: April 05, 2011;
Accepted: July 13, 2011

We examined the frequency and spectrum of podocin *NPHS2* mutations in Indian children with sporadic steroid resistant nephrotic syndrome (SRNS). Of 25 children screened, only one (4%) had a pathogenic mutation resulting in a stop codon. The allele and genotype frequencies of the four known single nucleotide polymorphisms detected in the cohort were similar to that of controls. This finding emphasizes the need to screen for mutations in other genes involved in the pathogenesis of SRNS.

Key words: India, *Nephrotic syndrome, Podocin, Steroid resistance.*

Published online: 1 November, 2011 PII: S097475591100155-2

Nephrotic syndrome is a common glomerular diseases in children [1]. Approximately 80% of all children with sporadic nephrotic syndrome respond to steroids. The remainder (20%) who do not respond after 4 weeks of steroid therapy are termed steroid resistant nephrotic syndrome (SRNS). These children are often resistant to immunosuppressive therapy and many progress to chronic kidney disease (CKD) requiring dialysis [2]. Recent molecular studies performed in children with sporadic primary SRNS have identified mutations in several genes encoding proteins involved in maintaining the integrity of glomerular filtration barrier [3]. Mutational analysis in SRNS would help in preventing unnecessary exposure to immuno-suppressants and their adverse effects, besides helping in prognostication. Mutations in the *NPHS2* gene, encoding podocin, are present in 6.4% to 30% of sporadic SRNS in different parts of the world [4-6]. Data from India on the prevalence of genetic mutations in children with SRNS is limited [4]. The aim of the study was to evaluate frequency and spectrum of podocin mutations in Indian children with sporadic SRNS.

METHODS

Children diagnosed with initial SRNS as defined by Indian Pediatric Nephrotic Group (IPNG) consensus guidelines were included after informed consent [7]. All included children had sporadic SRNS with follow up for at least 6 months. Patients with a family history of nephrotic syndrome, age of onset less than 6 months or with secondary nephrotic syndrome were excluded. The clinical data obtained from the patient record included demographic data, age of onset, response to immunosuppressive therapies, histological features on the kidney biopsy, progression to CKD and recurrence of nephrotic syndrome after renal transplantation. The response to therapy and staging of chronic kidney disease (CKD) are defined as per published guidelines [7,8].

To differentiate the mutations from polymorphisms, chromosomes from 50 healthy adults were screened as controls. Genomic DNA was extracted from peripheral blood leukocytes using standard laboratory protocol. All the eight exons of the *NPHS2* gene were amplified by using primers located on the intron-exon boundaries as described by Boute, *et al*. [9]. Primers for exon 3 and exon 6 were redesigned and are available on request. Direct sequencing of the amplified products was carried out using the Automated 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were evaluated for variants with the FinchTV software (ver 1.4.0, Geospiza, Inc USA). The frequencies for various SNPs were compared between cases and controls using chi-square and Fisher exact test (SPSS 16.0, SPSS Inc, Chicago,IL). P <0.05 was considered statistically significant.

WHAT THIS STUDY ADDS?

- *NPHS2* mutations were not found to be a major cause of steroid resistant nephrotic syndrome in the studied Indian children.

RESULTS

Twenty five children with sporadic SRNS (16 girls) were included in the study. The mean age (SD) at the onset of nephrotic syndrome was 3.5 (3.0) years (range 0.6 to 11 years). Parental consanguinity was found in 13% of the families. Kidney biopsy showed focal segmental glomerulosclerosis in 12, mesangial hypercellularity 6, and minimal change disease in 7 patients. Most children received multiple immunosuppressants with variable response. Initially, all patients had normal renal function. During follow up (range 0.6 to 9 years), 11 children (44%) maintained normal renal function (4 complete remission, 7 had persistent proteinuria), 3 (12%) developed CKD stages II – IV and 11 (44%) progressed to CKD stage V. The median duration for progression to CKD stage V from onset of disease was 1.9 years.

Screening for variations in NPHS2 gene: Only one patient (4%, 95% CI 3.8, 11.8) had a pathogenic mutation. The sequencing results showed the pathogenic mutation in exon 1 in one patient at nucleotide position 211, C>T resulting in a stop codon R71X. This child developed nephrotic syndrome at the age of 2 years and progressed to CKD stage V requiring maintenance hemodialysis by age of 6 years. In addition, four known single nucleotide polymorphisms (SNPs) were also detected in the cohort. The SNP, rs1079292 were detected in 8 and 7 patients and controls, respectively. The SNP, rs1410592 were observed in 22 patients and 40 controls. The frequencies of both the SNPs in the patients were not different from that observed in the control samples (P = 0.07 and 0.66, respectively). Two other SNPs, rs3738423 and rs3818587 were seen in one child each. One child with SRNS was detected to have all the four SNPs. None of the SNPs altered the podocin protein as the nucleotide substitution did not change the amino acid.

DISCUSSION

Molecular studies have implicated many genes like *NPHS2, NPHS1, WT1, ACTN4, CD2AP* and *TRPC6* in the pathogenesis of SRNS [1]. This study evaluated the frequency of *NPHS2* mutations in SRNS in Indian children. We found a homozygous mutation at nucleotide position 211, C>T in one patient, resulting in a stop codon R71X. This mutation in exon 1 leads to the formation of a truncated protein, rendering it non-functional. A similar mutation was reported in one patient in a Chinese pedigree study [10]. Detection of such mutations at an early stage, besides helping in prognostication, could prevent over-treatment with immunosuppressive drugs that are expensive and have side effects. We also identified four known polymorphic variants rs1410592, rs1079292, rs3738423 and rs3818587 and these did not affect the protein function. There was no significant difference in the allele and genotype frequency of these SNPs in the patients and the controls suggesting that the SNPs detected in patients did not increase the risk of nephrotic syndrome. The prevalence of NPHS2 mutations observed in this cohort of Indian children with sporadic SRNS is low (4%) and is similar to the Chinese (3%), Korean (0%), and the Japanese (0%) populations [1,3,11]. In contrast, the prevalence of mutations in NPHS2 is higher in Europe and North America affecting between 10.5-28% of the sporadic SRNS children (3-5). In conclusion, NPHS2 mutations do not appear to be a major cause of SRNS in Indian children; although, the study cohort was small and it is a major limitation of this study. We propose that mutations in other genes involved in pathogenesis of SRNS, in addition to podocin, should be screened in a larger population in order to plan a suitable genetic screening in these children.

Contributors: AV: designed the study, performed the data analysis, drafted the manuscript and will act as guarantor; AS and AR: performed the mutation analysis and literature review; TSS: contributed to critical evaluation of manuscript; KDP: contributed to study design and critical evaluation of manuscript.
Funding: Sarojini Damodaran Foundation and Nadathur Holdings; *Competing interests*: None stated.

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