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p.R138Q and p.R229Q Screening In NPHS2 Gene in a Moroccan Cohort with Steroid Resistant Nephrotic Syndrome

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ABSTRACT: Mutations in the *NPHS2* gene encoding podocin are implicated in an autosomal-recessive form of nonsyndromic steroid-resistant nephrotic syndrome in both pediatric and adult patients. The p.R138Q (c.413G>A) mutation in exon 3 was the most prevalent mutation in European series. The p.R229Q (c.686G>A) variant in exon 5 is the first human variant discovered with a mutation-dependent pathogenicity. We aimed in this study to screen for the p.R138Q mutation and the p.R138Q variant in a Moroccan cohort with Steroid Resistant Nephrotic Syndrome.

KEYWORDS: SRNS, NPHS2, p.R138Q, p.R229Q, FSGS

INTRODUCTION

Nephrotic syndrome (NS) is a clinically heterogeneous disease characterized by different histological variants (1–3) and genetic determinants (4,5). Clinically, it is divided into two forms based on the response to steroid therapy: steroid-sensitive and steroid-resistant (6). NS is characterized by heavy proteinuria, hypoalbuminemia, edema, and dyslipidemia. Although most patients are steroid-sensitive NS (SSNS), about 20% of children and 40% of adults are steroid-resistant NS (SRNS) and progress to end-stage renal disease (ESRD). In these cases, renal histology typically shows focal segmental glomerulosclerosis (FSGS) (7–9). Steroid resistant nephrotic syndrome (SRNS) remains one of the most intractable common causes of end-stage renal disease (ESRD) in children. Indeed 50 to 70% of these children developed end-stage renal disease within 5 to 10 years of diagnosis (10). In the era of high throughput sequencing, new candidate genes associated with monogenic and genetic heterogeneous diseases such as steroid resistant nephrotic syndrome (SRNS) pile up (11–16). However, mutations in the three main genes (*NPHS1*, *NPHS2*, and exons 8 and 9 of *WT1* gene) are still the most frequent molecular cause of SRNS in childhood and adolescence. The prevalence of mutations was inversely correlated with age of onset. The percentage of patients for whom a genetic cause was identified decreased from 69% in congenital SRNS (onset in 0-3 months) to 21% in patients with an age of onset between 19 and 25 years (15). Missense mutations represented the largest group of NPHS2 mutations with a total of 53 different mutations described (42%). The p.R138Q (c.413G>A) mutation in exon 3 was the most prevalent mutation in European series, likely due to a founder effect in Northern Europe (17). And was detected in 32% and 44%, respectively, of all affected NPHS2 alleles in two large European series (18,19). This arginine residue at position 138 is highly conserved among the stomatin-like protein family members and is crucial for podocin function (5). The p.R229Q (c.686G>A) variant in exon 5 is the first human variant discovered with a mutation-dependent pathogenicity: it is only pathogenic when trans-associated to specific mutations ([p.R229Q];[mut]pat) (20). These mutations exert a dominant negative effect on p.R229Q podocin by blocking its membrane trafficking through an altered hetero-oligomerization (20,21).

The aim of this study was to screen for those two mutations by direct sequencing of the exons 3 and 5 of the NPHS2 gene on a Moroccan cohort of 21 patients with SRNS.

MATERIALS AND METHODS

Patients

Twenty one Moroccan patients from 21 unrelated families whose clinical diagnosis was in favor of SRNS were enrolled in this study. Patients were referred from the nephrology and pediatric departments of Hassan II University Hospital of Fez. The study was approved by University Hospital Ethics Committee (Faculty of Medicine and Pharmacy, Fez). Each patient was informed about the aim of the study, and the consent to genetic testing was obtained.

Molecular approach

Genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Inc.). Molecular study was performed by direct sequencing of exons 3 and 5 of NPHS2 gene to investigate the most prevalent mutation in Europe p.R138Q (c.413G>A) and the first human variant discovered with a mutation-dependent pathogenicity p.R229Q (c.686G>A). All studied exons (coding regions and exon-intron junctions) of the NPHS2 gene were amplified by PCR. The PCR conditions were carried out in a volume of 25 mL containing 100ng of DNA, PCR (buffer 10×), 0.2 mM of each dNTP, 1.5 mM MgCl2, 1 µL of each primer, and 0.5U Taq DNA polymerase (Invitrogen). All PCR primers and conditions are shown in (Table 1). PCR was performed in a thermocycler, which was arranged uniformly for all reactions as followed: initial denaturation step at 95°C for 1 min, denaturation step at 94°C for 30 s, annealing step differs according to the exon (58°C for exon 3and 56°C for exon 5) for 1 min, polymerization step at 72°C for 1 min, and final extension step at 72°C for 9 min. PCR products were then revealed by electrophoresis in a 2% agarose gel with ethidiumbromide. Mutation screening was performed by direct sequencing using the BigDye Terminator Kit (Applied Biosystems: ABI) on an ABI 3500Dx Genetic Analyzer v2.3 and the SeqA v5.4 software (Applied Biosystems: ABI). Obtained sequences were then aligned via NCBI Nucleotide BLAST tool.

Table1. Oligonucleotide Primers Used for Polymerase Chain Reaction

Gene		Forward	Reverse	Amplicon length (bp)	Annealing temperature (C)
NPHS2	Exon 3	TTCTGGGACTGATTTGAAAG	TGAAGAAATTGGCAAGTCAG	167	58
	Exon 5	CATAGGAAAGGAGCCCAAGA	TTCAGGCATATTGGCCATTA	292	56

RESULTS

Our study was composed of 21 patients from 21 unrelated families from different regions of Morocco: 9 female and 12 male patients with a sex ratio M/F of 1.3. Patients were aged from 3 to 61 years with a mean age of 19 years and 12 (57%) were ≤16 years. Molecular diagnosis was performed by direct sequencing of exons 3 and 5 of NPHS2 gene to investigate the p.R138Q (c.413G>A) mutation and the p.R229Q (c.686G>A) variant respectively. Analysis of the studied exons (coding regions and exon-intron junctions) shows the absence of pathogenic mutations.

DISCUSSION

NPHS2 encodes the podocyte slit diaphragm protein podocin, which plays an important role in maintaining the filtration barrier. Mutations in the NPHS2 gene have been reported to account for a significant proportion of all nephrotic patients. They correspond roughly to 45–55% of familial forms and 8–20% of sporadic disease, with variations according to the different patient cohorts and the different sub-phenotypes studied (22). The incidence of familial podocin mutations in our population represented only 7.6%, with childhood onset NS (23), similar to that in Libyan and Tunisian families (7.6%) (1), lower than in Turkish (29.2%) (24), European, and American children (40%) (25), but higher than in African–American (26), Japanese, and Korean children (0%) (27,28). In most patients, the culprit mutations are located in exons 1 to 6 of NPHS2. Age of onset in these patients is 1.1 (range, 0-13.7) years, and kidney failure is reached at 8.0 (range, 0-26) years (6). Mutations in NPHS2 can also cause nephrotic syndrome with onset in adulthood (29). In the majority of these patients, a heterozygous rare pathogenic variant in NPHS2 is observed in association with heterozygosity for R229Q (6,30). Median age of onset of nephrotic syndrome for patients with the R229Q variant and a pathogenic variant is 19 (range 0-39) years. Median age at onset of kidney failure for these patients is 27.9 (range, 9.3-43.5) years (6). Compared to the allele frequency of 3.7% in the European population for the R229Q variant, the frequency is lower in Asian (0%-2.7%), African (0.7%), and Latino (0.9%) populations (31), Which can explain its absence in our cohort and in the Tunisian cohort (1), even if the small size of our cohort could be a limiting factor. In an Egyptian study, NS patients showed significant higher frequency of heterozygous GA genotype of p.R229Q (89.5%) compared to control group (10.5%), and increased risk of NS. Significant increase of heterozygous genotype (GA) was observed in SRNS (68.2%) as compared to SSNS patients (6.5%) (32).

CONCLUSION

The limitation of our study is the size of our cohort. Nevertheless, SRNS is a rare disease. For more general conclusions, our results need to be confirmed in a larger sample of patients.

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