CHILDHOOD NEPHROTIC SYNDROME RESPONSE TO STEROID THERAPY AND THE RELATIONSHIP TO NPHS2 GENE MUTATION: A MULTICENTER STUDY IN SAUDI ARABIA

Burhan Edrees

ABSTRACT

Background: Nephrotic syndrome (NS) is an expression of many glomerular diseases and not a disease in itself. Based on response to steroids, NS is categorized into steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS). SRNS patients are at a high risk of developing end-stage renal disease. The aim of the study was to evaluate steroid response and the prevalence of NPHS2 gene mutations in Saudi children with NS. Mutations in the NPHS2 podocin gene on chromosome 1 are the cause of a familial, autosomal recessive SRNS with the most frequently reported variant R229Q.

Materials & Methods: The study involved 74 Saudi children with NS, selected (by pediatric nephrology consultants) in 2014 from the pediatric nephrology clinics of different hospitals across regions in Saudi Arabia. Intravenous blood samples were drawn in sterile tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic DNA samples were isolated and PCR products were assessed by restriction enzyme digestion to identify R229Q and R291W mutations. Positive results derived from DNA capillary sequencing analysis confirmed R229Q mutation.

Results: Of the 74 children, 34.3% had SRNS. Of these, only 27% had a positive family history of this condition. Within the NPHS2 gene, we found one R229Q missense mutant allele. R291W mutation was absent.

Conclusion: We identified an R229Q mutation in a lower frequency compared with other ethnic populations. However, one has to screen more genetic loci within the NPHS2 gene. Other responsible genes, for example, NPHS1, NPHS3, WT1, and ACTN4, could be investigated for this purpose.

Keywords: Nephrotic syndrome, NPHS2, steroid resistance, R229Q

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INTRODUCTION

Nephrotic syndrome (NS) is an expression of many glomerular diseases and not a disease in itself. These diseases may be acute and transient, such as post-infectious glomerulonephritis, or chronic and progressive, such as focal segmental glomerulosclerosis (FSGS)[1]. NS is characterized by the presence of immense proteinuria, hypoalbuminemia, edema, and hyperlipidemia. NS is categorized with regard to response to steroids into steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS). SRNS patients are at a high risk of developing end-stage renal disease[2]. Mutations in the NPHS2 podocin gene on chromosome 1 are the cause of a familial, autosomal recessive SRNS form of FSGS with the most frequently reported variant R229Q[3, 4]. Podocytes are responsible for preventing plasma proteins

Correspondence: Burhan Edrees (MD), Associate Professor, Department of Pediatrics, Umm Al-Qura University, Saudi Arabia. Phone: 00966505713308. Email: bmedrees1956@gmail.com

from being filtered into the urinary space. Any loss or injury in podocytes leads to proteinuria. Podocin interacts with the intracellular domain of nephrin. It is an integral membrane protein and belongs to the stomatin superfamily. Reduced podocin protein levels have been detected in the glomeruli of PA-nephrotic rats[5]. So far, podocin has been shown to be expressed exclusively in podocytes and has been localized to the SD; it also interacts with CD2AP[6, 7]. Podocin has been shown to be anchored in lipid rafts, which are microdomains rich in sphingolipids and cholesterol, in the outer leaflet of the plasma membrane[8, 9]. This study was aimed at evaluating steroid response and the prevalence of NPHS2 gene mutations in Saudi children with NS.

MATERIALS & METHODS

Subjects

After taking consent from parents, 74 Saudi children with NS were selected for the study in 2014. They were selected (by pediatric nephrology consultants) from the pediatric nephrology clinics of different hospitals across regions in Saudi Arabia (for example, Makkah, Jeddah, Al-Hada, Al-Taif, Riyadh and Eastern regions). Selection was based on clinical criteria and done in a manner to ensure there was at least one affected child from each region. The children aged from neonates to 18-year olds.

Sample Collection and Storage

Intravenous blood samples were drawn in 3.5 cm^3 sterile tubes containing ethylenediaminetetraacetic acid (EDTA) solution. The blood samples were transferred to the Molecular Laboratories Department of Medical Genetics, Faculty of Medicine (Umm-Al-Qura University), in ice blocks at a temperature of around 1–10°C and stored in a freezer at -20°C until use.

Molecular Study

DNA isolation: The blood-EDTA samples were thawed, and genomic DNA samples were isolated using the QIAamp DNA Blood Mini Kit (supplied by Qiagen, USA), as recommended by the manufacturer. Proteinase K (20 µl) was pipetted into the bottom of a 1.5 ml microcentrifuge tube. A whole blood sample (200 µl) was added to the microcentrifuge tube. We used up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5x106 lymphocytes in 200 µl phosphate-buffered saline (PBS). Lysis buffer (AL; 200 µl) was added to the sample and incubated at 56°C for 10 minutes for complete hemolysis of RBCs, lysis of cell pellet and digestion of proteins. Absolute ethanol (200 µl) was mixed with the sample to precipitate the DNA. The sample ethanol was carefully applied to the QIAamp spin column (QIAGEN, USA), and the mixture was centrifuged at 8,000 rpm for one minute. The filtrate was discarded. The column was carefully washed with the washing buffer (AW1; 500 µl). The tube was centrifuged at 8,000 rpm. Another 500 µl of the washing buffer (AW2) was added and again centrifuged at full speed for two minutes. The column was opened and 200 µl buffer AE (elution buffer) was added, incubated at room temperature for one minute and then centrifuged at full speed for one minute. The highly pure DNA sample was refrigerated at 4°C until use; it can be stored at 20°C for a longer time and at 70°C forever.

Detection of R229Q and R291W mutations within NPHS2 gene: To identify R229Q and R291W mutations within the NPHS2 gene, oligonucleotide primers were used: 5`-AGG ATT TAC CAC AGG ATT AAG TTG TGC A-3` and 5`-TAG CTA TGA GCT CCC AAA GGG ATG G-3` for exon 5, and 5`-GAG GCT TGC AAG TCT GTG TGA AAG C-3` and 5`-AGG AAG CAA AGG GGA AAT GTT CTC C-3` for exon 7.

The PCR products were assessed by restriction enzyme digestion from all available family members. ClaI digestion of exon 5 PCR products (545-bp) normally produces two fragments of 364- and 181-bp. An R229Q mutation (G>A transition at nucleotide 755) was detected by the loss of the ClaI digestion site. An R291W mutation (C>T transition at nucleotide 941) created a new PflMI digestion

site and was detected by PflMI digestion of exon 7 PCR products (283-bp) into two fragments (155- and 128-bp).

All PCR products and enzymatic digestion products were visualized using ethidium bromide staining under the UV gel documentation system.

Sequencing analysis: DNA capillary sequencing analysis using the BigDye terminator method (supplied by US-based Applied Biosystem Inc., Model 5300) gave positive results, confirming the R229Q mutation.

RESULTS

Of the total number of subjects, 34.3% had SRNS, while 65.7% had SSNS. Among SRNS patients, 27% had a positive family history, while among SSNS subjects, 19% had a positive family history of the condition.

Within the NPHS2 gene, we found one R229Q missense mutant allele due to G>A transition at nucleotide 755 in exon 5, predicting an Arg>Gln substitution. This compound heterozygote case was associated with a female suffering from an idiopathic nephrotic syndrome. However, the allele frequency of R229Q for all Saudi nephrotic cases was 0.68% (1/148 cases). We confirmed R229Q mutation by DNA capillary sequencing analysis using the BigDye terminator method.

Figure 1. A-forward revealed alignments of DNA sequencing electropherogram of NPHS2 gene (exon 5) for a female with an idiopathic nephrotic syndrome showing an R229Q mutation (G>A; R may be A or G, Y may be C or T)

A-Forward strand:

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Query 1    ATCGTGACCAAAGACATGTTTATAATGGAGATAGATGCCATTTGCTACTACCGAATGGAA  60
Sbjct 179  ATCGTGACCAAAGACATGTTTATAATGGAGATAGATGCCATTTGCTACTACCGAATGGAA  238

Query 61   AATGCCTCTCTTCTCCTAAGCAGTCTTGCTCATGTATCTAAAGCTGTGCAATTCCTTGTG  120
Sbjct 239  AATGCCTCTCTTCTCCTAAGCAGTCTTGCTCATGTATCTAAAGCTGTGCAATTCCTTGTG  298

Query 121  CAAACCACTATGAAGCGTCTCCTAGCACATCGATCCCTCACTGAAATTCTTCTAGAGAGG  180
Sbjct 299  CAAACCACTATGAAGCGTCTCCTAGCACATCRATCCCTCACTGAAATTCTTCTAGAGAGG  358

Query 181  AAGAGCATCGCCCAAGATGCAAAG  204
Sbjct 359  AAGAGCATCGCCCAAGATGCAAAG  382

CGA > CAA
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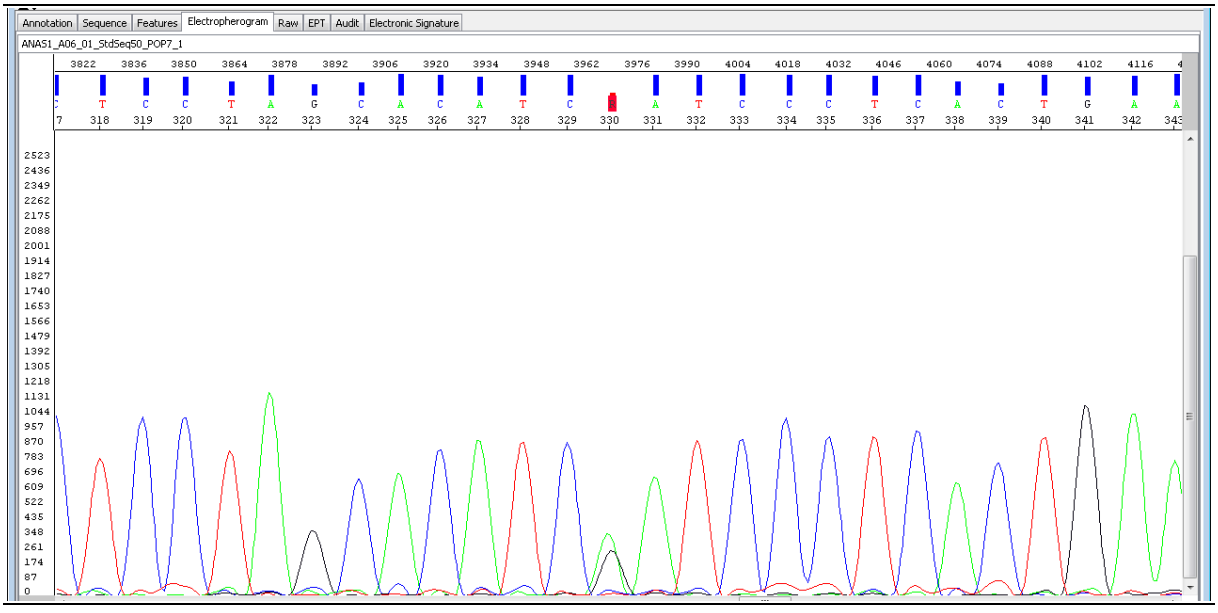
Figure 2. Electropherogram presentation (reverse strand) showed R229Q (exon 5, G > A)



DISCUSSION

In this study, we identified an R229Q mutation in a lower frequency compared with other ethnic populations. In a review conducted by Franceschini *et al.* that included five old studies, the R229Q variant was not found to be associated with focal segmental glomerulosclerosis in the US population of African descent[10]. In contrast, the R229Q variant was found to be associated with an increased risk (20–40%) of focal segmental glomerulosclerosis in the population of Europeans[10]. In our study, in the NPHS2 gene, we found one mutant R229Q allele with a frequency of 1/148 alleles (0.68%) in Saudi NS cases. For different ethnic populations, it has been reported that screening of R229Q mutation revealed the following allele frequencies: 1.6% (1 of 64 alleles from individuals of African descent), 3.1% (3 of 98 alleles from Brazilian individuals), 3.6% (9 of 248 alleles from a DNA panel dominated by North American and Western European ethnicities), and 5.8 (6 of 104 alleles from North American individuals). However, it is clear that the allele frequency of R229Q mutation within the NPHS2 gene among Saudi nephrotic cases is much lower than that in other ethnic populations. Another study by Cho and colleagues showed that the incidence of NPHS2 mutations seemed to be very rare in Korean children. No R219W mutations were present in this study.

Finally, we recommend that further genetic loci within the NPHS2 gene should be screened in Saudi children. Other responsible genes, for example, NPHS1, NPHS3, WT1, and ACTN4, could be investigated for this purpose.

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