Nphs2 Mutations in Familial Steroid Resistant Nephrotic Syndrome in Gaza

Fadel A. Sharif*1, Mustafa El-Aila2, Mohammed J. Ashour1, Shadi J. Al-Ashi1, Ata J. Deif Allah1

1Medical Laboratory Sciences, The Islamic University of Gaza, Palestine
2The Rantisi Children Hospital of Gaza, Palestine

Corresponding author: Fadel A. Sharif, Department of Medical Laboratory Sciences, Islami University of Gaza, Gaza, Palestine
E-mail: fsharif@iugaza.edu.ps, fadelsharif@gmail.com

Citation: Fadel A. Sharif et al. (2017), Nphs2 Mutations in Familial Steroid Resistant Nephrotic Syndrome in Gaza. Int J Nep & Uro Dis. 1:1, 27-30. DOI: 10.25141/2577-0152-2017-1.0027

Received: December 18, 2017; Accepted: December 21, 2017; Published: December 30, 2017

Abstract

Background: Nephrotic syndrome (NS) is characterized by edema, massive proteinuria, hypoaalbuminemia, hyperlipidemia, and may progress to end stage renal disease. Based on the response to steroid therapy, NS is categorized as steroid-sensitive or steroid-resistant (SRNS). SRNS is inherited as an autosomal recessive disorder with NPHS2 being the most frequently mutated gene. NPHS2 encodes the glomerular protein podocin, which has an important role in controlling slit membrane permeability and glomerular ultrafiltration.

Objective: The spectrum and frequency of NPHS2 mutations in Palestine have not been explored before. The aim of this study is to identify NPHS2 pathogenic mutations associated with SRNS in Palestinian families.

Methods: Twenty SRNS-diagnosed patients were recruited from The Rantisi Children Hospital of Gaza. All the eight exons of the NPHS2 gene were PCR-amplified from patients genomic DNAs using primers located on the intron-exon boundaries. Direct sequencing of the purified PCR fragments was then ensued by automated Sanger sequencing method. Nucleotide changes were verified by comparing obtained sequences with the reference gene sequence stored in the NCBI database.

Results: Analysis of the obtained sequences identified previously known mutations in 3 (15%) of the patients. Two of the mutations, p.G130K and p.R229Q were missense mutations and the third, p.R138X was a nonsense mutation. All mutations were present in homozygous form.

Conclusions: The current study reports the identification of p.G130K, p.R138X, and p.R229Q NPHS2 mutations in SRNS patients in Gaza-Palestinian. The mutations thus identified would spare patients from the unnecessary and harmful immunosuppressive steroids and help physicians and patients’ families take proper decisions regarding patient management and their future offspring.

Keywords: SRNS, NPHS2, Sequencing, Gaza-Palestine.

Introduction:

Nephrotic syndrome (NS) is considered as one of the main kidney diseases in children. Up to 20% of afflicted children do not respond to steroid therapy and their disorder is termed steroid-resistant nephrotic syndrome “SRNS” [1,2].

Molecular studies performed on SRNS patients have identified mutations in several genes encoding proteins involved in maintaining the integrity of glomerular filtration barrier. However, pathogenic mutations in three main genes (NPHS1, NPHS2, and WT1) represent the most frequent molecular cause of SRNS [3,4]. NPHS2 gene (OMIM 604766) encodes podocin that is localized at the slit membrane in Bowman’s capsules in the nephrons of the kidney [5]. Podocin interacts directly with nephrin. Podocin and nephrin are key components of the slit diaphragm of the glomerular epithelial cell and are essential for the normal function of the glomerular filtration barrier. Podocin deficiencies alter slit diaphragm permeability and can also alter the processing and localization of nephrin [6]. NPHS2 gene is located at chromosome 1q25–31 and contains 8 exons coding for the 383 amino acid long podocin. So far, more than 200 NPHS2 mutations are registered in the HGMD (http://www.hgmd.cf.ac.uk) database. The mutations are distributed along the entire coding region of the gene and cause all kinds of sequence alterations [7]. Mutational analysis in SRNS would spare the patients from the unnecessary exposure to immuno-suppressants and their adverse effects, besides helping in identifying patients and families who...
can benefit from kidney transplant and prenatal/preimplantation diagnosis programs. This study was conducted to evaluate the spectrum and frequency of NPHS2 mutations prevalent in Palestinian residents of Gaza strip. Twenty families having at least one SRNS child were enrolled in the study. The eight exons and intron-exon boundaries of the NPHS2 gene were examined by direct sequencing.

**Methods**

**Study subjects**
The population of this mutation analysis study consisted of 20 (10 girls and 10 boys) SRNS patients. The subjects were recruited from The Rantisi Children Hospital of Gaza. All the patients were under 12 years of age.

**Ethical considerations**
Informed consent was taken from all the patients parents. The objective and benefits of the study were fully explained to the participating families. The study was approved by the local ethics committee and was in accordance with the principles of the 2000 Helsinki declaration.

**Mutation Analysis**
Anticoagulated venous blood (~2 ml) was collected from the participating families members. Genomic DNA was prepared from peripheral blood lymphocytes using Wizard Genomic DNA Purification Kit (Promega, USA) according to the manufacturer’s protocol. The 8 exons of the NPHS2 gene and their exon/intron boundaries were amplified using the PCR primers and protocols reported by Boute et al. [8]. The PCR products purified by a commercial purification kit (Invitrogen, Germany) were directly sequenced in both directions using the Big Dye Termination kit (Applied Biosystems, USA) according to manufacturer’s instructions. Obtained NPHS2 sequences were analyzed for nucleotide changes by comparing them to the reference sequences (NPHS2, NM_001297575.1) stored in the NCBI database (NCBI human genome build 38.7) using the NCBI BLAST (basic local alignment search tool) tool. Detected sequence changes were then searched for novelty using the NPHS2 variation table stored in the Ensembl (http://www.ensembl.org) database.

**Results**
The study cohort consisted of 20 children (10 girls and 10 boys) suffering from SRNS. The mean age at onset of proteinuria was 3.57 years (range, 2 month to 6 years). Three (15.0%) patients had positive family history (i.e., familial NS) and 17 (85.0%) had negative family history (i.e., sporadic NS).

**Detected Mutations**
Table 1 below summarizes the 3 mutations detected in the study subjects. The first mutation c.388G>A (in exon 3) was detected in one patient where he was homozygous for this mutation. Consultation of the Ensembl variant table for NPHS2 gene mutation showed that this is a previously known pathogenic mutation. This nucleotide alteration leads to a missense mutation; replacing a normal glutamic acid at position 130 with lysine (i.e., p.E130K) in the podocin polypeptide.

The second SRNS patients harbored the transition mutation (c.412G>T). This exon 3 pathogenic nonsense mutation has been described before. The patient was homozygous for this mutation which introduces a premature stop codon in place of the amino acid arginine at position 138 (p.R138X) in the gene product. The third mutation identified in our cohort of SRNS patients was the transition (c.686G>A) in exon 5. This previously reported nonsense mutation replaces the amino acid arginine at position 229 by glutamine (p.R229Q). This mutation was also evident in homozygous form.

| Patient ID number | Exon | Nucleotide change | Effect on protein | Genotype | Proteinuria onset (Month) |
|-------------------|------|------------------|-------------------|----------|--------------------------|
| 7                 | 3    | c.388G>A         | p. Glu130Lys      | Homozygous | 2                        |
| 17                | 5    | c.686G>A         | p. Arg229Gln      | Homozygous | 45                       |
| 21                | 3    | c.412C>T         | p. Arg138X        | Homozygous | 60                       |

**Discussion**
Identification of mutations associated with genetic disorders is important because it clarifies the cause of the disease, influences physicians’ decision on patient treatment and management, and helps the patient’s family take very important decisions for their future progeny. Moreover, as in the case of SRNS, patients harboring pathogenic mutations can be spared the side effects of useless immunosuppressive therapy and could be considered for kidney transplantation. This study, the first of its kind in Palestine, was conducted in order to identify NPHS2 gene mutations in 20 SRNS patients residing in Gaza-Palestine. Based on our results, the incidence of podocin mutations in the examined cohort approached 15%. This figure is comparable to the NPHS2 mutation detection rate reported from studies on Turkish and Brazilian SRNS children [5,9] but lower than that documented in European and American children [9-12]. Interestingly, no pathogenic NPHS2 mutations were found in Japanese and Korean SRNS children [9]. This controversy could be due to the high heterogeneity in the nephrotic syndrome and the associated genetic loci (so far more than 40 loci have been implicated in childhood nephrotic syndrome [13] and reflects ethnic differences in disease-associated genes. In the SRNS patients examined in this study we could identify three pathogenic genotypes (all in homozygous form) comprising three
different NPHS2 mutations (p.E130K, p.R138X, and p.R229Q) in 3 out of the 20 investigated SRNS patients. It is important to indicate here that all the three affected children were the offspring of consanguineous parents and had positive family history for NS. The first mutation (p.E130K) was encountered in homozygous form in a male patient. This missense mutation results from a G to A transition in the coding region of exon 3 at position 388. The mutation was also evident in the affected brother of the patient. The mutation replaces an acidic amino acid (glutamic acid) with a basic amino acid (lysine) in the podocin polypeptide. Obviously, the two amino acids have different characteristics and this alteration is expected to impair the function of the podocin. Moreover, this amino acid is among the amino acids located in the PHB (prohibitin homology). PHB encompasses the amino acids 125 to 284 and is required for the binding of podocin to nephrin [14]. This mutation was reported before in a homozygous form in two patients of Arabian origin [15]. The familial and autosomal recessive nature of this mutation was confirmed by detecting the same genotype in the affected brother of the patient and in his carrier parents.

The second mutation (p.R138X) was detected in homozygous form in a female patient. This transition mutation is due to a C to T transition at nucleotide 412 in exon 3. The mutation was also evident, also in homozygous form, in the affected sister of the patient and in the carrier parents. The genetic alteration in this mutation introduces a premature (TGA) stop codon in place of the amino acid arginine at position 138 in the podocin sequence thus leading to the production of a non-functional truncated polypeptide. This amino acid is also located in the PHB domain of podocin and was shown to be crucial for the function of podocin [14]. p.R138X mutation was first described by Boute et al. [9]. Interestingly, this mutation has been shown by Frishberg et al. [16] to be prevalent among Arab SRNS patients (where 15 out of their 27 patients i.e., 55% harbored this mutation in homozygous form) a result which made the authors raise the possibility of this mutation as being a founder mutation among Arabs [16]. This same mutation was also reported by Cohen et al. (2007) in homozygous form, again in a girl of Arabic descent [17], further potentiating the importance of this mutation in patients of Arab ethnicity.

The third mutation p.R229Q was documented in homozygous form in a female patient who also has an affected sister harboring the mutation. This missense mutation occurs as a result of G to A transition at nucleotide 686 in exon 5 and leads to replacing the amino acid arginine by glutamine. Exon 5 also constitutes part of the PHB domain and the missense mutation is expected to influence the function of podocin. This controversial genetic change (c.686G>A) is the most frequently encountered mutation in diverse populations [9]. Based on the conservation of Arg 229 residue in podocin homologs, segregation of the mutation with the disease, and functional study of the mutated podocin made Tsukaguchi et al. (2002) consider this relatively common genetic change as a disease-causing mutation [18].

In addition to the three identified pathogenic mutation, sequencing data revealed four previously known benign polymorphisms (results not shown). All the polymorphisms were detected in heterozygous form.

In conclusion, mutations in the NPHS2 gene contribute to familial SRNS in Palestine. Mutations in this gene, exons 3 and 5 in particular, should be searched for in SRNS children in order to avoid unnecessary immunosuppressive steroid that may escalate patient morbidity. Identification of the responsible mutations will also help the affected families in planning for kidney transplant, carrier screening, prenatal and even pre-implantation genetic diagnosis. Further genetic work should be done for NPHS2-negative families in order to identify potential mutations in other genes (e.g., NPHS1 and WT1) strongly implicated in the pathogenesis of SRNS.

Conflicts of interest

The authors declare no competing interests in this work.

References

1. Otukesh H., Otukesh S., Mojtabahzadeh M., Hoseini R., Fereshtehnejad S.M., et al. Management and outcome of steroid-resistant nephrotic syndrome in children. Iran J. Kidney Dis. 2009; 3(4): 210-217.

2. Patrakka J., Lahdenkari A.T., Koskimies O., Holmberg C., Warthiovaara J., Jalanko H. The number of podocyte slit diaphragms is decreased in minimal change nephrotic syndrome. Pediatric Research, 2002; 52(3): 349-355.

3. Lipska B.S., Balasz-Chmielewska I., Morzuch L., Wasielewski K., Vetter D., et al. Mutational analysis in podocin-associated hereditary nephrotic syndrome in Polish patients: founder effect in the Kashubian population. J. Applied Genetics, 2013; 54(3): 327-333.

4. Guaragna, M.S., Lutaif A.C., Maciel-Guerra A.T., Belangero V., Guerra-Júnior G., De Mello M.P. NPHS2 Mutations: A Closer Look to Latin American Countries. BioMed Research International, 2017. doi:10.1155/2017/7518789.

5. Guaragna M.S., Lutaif, A.C., Piveta C.S., Souza M.L., de Souza S.R. et al. NPHS2 mutations account for only 15% of nephrotic syndrome cases. BMC medical genetics. 2015; 16(1): 88.

6. Otukesh H., Ghazanfari B., Fereshtehnejad S.M., Bakhshayesh M., Hashemi M., et al. NPHS2 mutations in children with steroid-resistant nephrotic syndrome. Iran J Kidney Diseases, 2009; 3(2).

7. Bouchireb K., Boyer O., Gribouval O., Nevo F., Huynh-Cong E., et al. NPHS2 Mutations in Steroid-Resistant Nephrotic Syndrome: A Mutation Update and the Associated Phenotypic Spectrum. Human mutation, 2014; 35(2):178-186.

8. Boute N., Gribouval O., Roselli S., Benessy F., Lee H., et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nature genetics, 2000; 24(4):349.

9. Kitamura A., Tsukaguchi H., Iijima K., Araki J., Hattori M., Ike-E., et al. NPHS2 Mutations in Steroid-Resistant Nephrotic Syndrome. Int J Nep & Uro Dis. 1:1, 27-30.

10. Caridi G., Perfumo F., Ghiggheri G.M. NPHS2 (Podocin) Mutations in Nephrotic Syndrome. Clinical Spectrum and Fine Mechanisms. Pediatric research, 2005; 57(5): 54R.
11. Weber S., Gribouval O., Esquivel E.L., Morinière V., Tête M.J., et al. NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. Kidney International, 2004; 66(2): 571-579.

12. Caridi G., Bertelli R., Di Duca M., Dagnino M., Emma F., et al. Broadening the spectrum of diseases related to podocin mutations. J. American Society of Nephrology, 2003; 14(5): 1278-1286.

13. Ha T.S. Genetics of hereditary nephrotic syndrome: a clinical review. Korean J. Pediatrics, 2017; 60(3):55-63.

14. Huber T. B., Simons M., Hartleben B., Sernetz L., Schmidts M., et al. Molecular basis of the functional podocin–nephrin complex: mutations in the NPHS2 gene disrupt nephrin targeting to lipid raft microdomains. Human Molecular Genetics, 2003; 12(24):3397-3405.

15. Sadowski C. E., Lovric S., Ashraf S., Pabst W. L., Gee H. Y., et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J. American Society of Nephrology, 2015; 26(6):1279-1289.

16. Frishberg Y., Rinat C., Megged O., Shapira E., Feinstein S., Raas-Rothschild A. Mutations in NPHS2 encoding podocin are a prevalent cause of steroid-resistant nephrotic syndrome among Israeli-Arab children. J. American Society of Nephrology, 2002; 13(2): 400-405.

17. Becker-Cohen R., Bruschi M., Rinat C., Feinstein S., Zennaro C., et al. Recurrent nephrotic syndrome in homozygous truncating NPHS2 mutation is not due to anti-podocin antibodies. American J Transplantation, 2007; 7(1): 256-260.

18. Tsukaguchi H., Sudhakar A., Le T. C., Nguyen T., Yao J., et al. NPHS2 mutations in late-onset focal segmental glomerulosclerosis: R229Q is a common disease-associated allele. The J. Clinical Investigation, 2002; 110(11): 1659.