Case Report

A novel mutation in *NPHS2* causing nephrotic syndrome in a Saudi Arabian family

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Abstract

We report a consanguineous family from Saudi Arabia with three affected children presenting with infantile nephrotic syndrome. In order to provide a molecular diagnosis, a genome-wide SNP analysis of the affected patients was performed. We identified a region of homozygosity on chromosome 1, containing the *NPHS2* gene. Direct sequencing, by exon PCR, of *NPHS2* identified a homozygous nucleotide change 385C>T within exon 3 in the three affected children, leading to a premature stop codon (Q129X). This homozygous truncating mutation in *NPHS2* is novel and was associated with a severe clinical phenotype. Additional mutations in related genes *NPHS1*, *PLCE1* and *NEPH1* were not identified, excluding tri-allelism within these genes in this family.

Keywords: homozygosity; nephrotic syndrome; *NPHS2*; podocin

Background

Nephrotic syndrome (NS) is a clinical diagnosis, characterized by oedema, proteinuria and hypoalbuminaemia. Congenital NS (CNS) manifests *in utero* or up to the first 3 months of life, while later-onset disease, between 3 months and 12 months of age, is termed infantile NS. When the clinical response of the patient is resistant to steroid therapy, it is termed steroid-resistant nephrotic syndrome (SRNS). Recent molecular genetic studies have identified several genes implicated in NS. These include *NPHS1*, *NPHS2*, *PLCE1*, *WT1*, *LAMB2* and *LMX1B*.

Genetic studies have identified that the *NPHS2* gene mutations may present as CNS in European [1,2] and Japanese patients [3]. However, clear genotype/phenotype correlations often cannot be made given that in some patients, both *NPHS1* and *NPHS2* mutations may be present, resulting in a tri-allelic genetic abnormality [1,2,4]. More recent studies have suggested that the most frequent genetic cause of congenital and infantile NS is mutations in *NPHS2*, which account for ~40% of cases in a European cohort [5].

NPHS2 mutations have also been associated with SRNS, which may be both familial and sporadic. In a Turkish study, *NPHS2* mutations were detected in ~30% of patients presenting with familial SRNS, the majority of whom had focal glomerulosclerosis on renal biopsy [6]. Thus, *NPHS2* mutations can present as nephrotic syndrome at birth, during early childhood years and later, including into adult life [7,8]. A widening of the phenotypic spectrum, from congenital to childhood NS, has also been noted for *NPHS1* mutations [9].

NPHS2 encodes the slit diaphragm protein, podocin, which interacts with nephrin and CD2AP [10], and is part of a protein complex within podocytes that includes Neph1 and Neph2 proteins.

In this report, we present a consanguineous Saudi Arabian family with three affected children with infantile NS. Each of the affected children were homozygous for a single nucleotide mutation 385C>T within *NPHS2*, leading to a premature stop codon (Q129X) and a predicted severely truncated podocin protein. Each parent was heterozygous for this mutation. This truncating mutation within *NPHS2* correlates with the severe clinical phenotype of infantile NS seen in the affected individuals.

Case report

The index case (IV:1, Figure 1A) presented at the age of 5 months of age, with nephrotic syndrome. Serum creatinine was 23 umol/L, with an estimated (Swartz formula [11]) GFR of 130 mL/min/1.73 m^2. Serum albumin was 18 mg/dL, and urine dipstick confirmed proteinuria and haematuria. Urine protein was quantified to be 2700 mg/m^2 per day. A renal USS showed slightly enlarged kidneys, and a renal biopsy revealed a mesangioproliferative glomerulonephritis. The child had an incidental finding of a cystic adenomatoid malformation of the lungs. The parents were healthy, with no proteinuria, were from southern Saudi Arabia, and were noted to be first cousins.

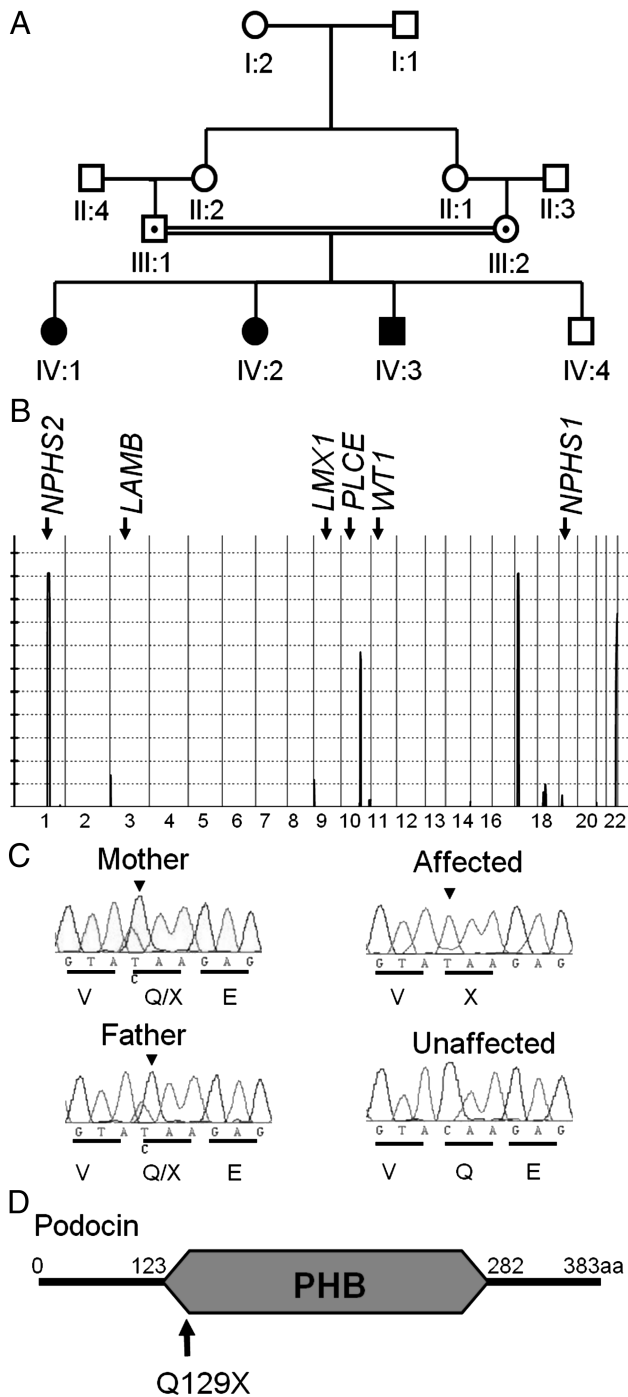Treatment was commenced with high-dose oral steroids, but the child did not respond. By 1 year of age, serum cre-

Fig. 1. (**A**) Pedigree of the Saudi Arabian family. Affected individuals are shown in shaded symbols. (**B**) Genome-wide homozygosity mapping in the affected individuals, annotated with candidate genes. (**C**) DNA chromatograms of the parents, affected and unaffected siblings of exon 3 in *NPHS2*. (**D**) Schematic representation of the 383 amino acid (aa) podocin protein, which includes a prohibitin homologues domain (PHB). The Q129X mutation is shown.

atinine was preserved (≤30 umol/L), while serum albumin remained low (17 mg/dL), and proteinuria persisted. Therapy was augmented with the addition of cyclosporin 12.5 mg twice daily, captopril 12.5 mg twice daily and furosemide 5 mg daily. The nephrotic syndrome failed

to respond to these measures. The clinical condition of the child worsened by the age of 30 months with significant facial and peripheral oedema, a persistently low serum albumin (13 mg/dL) and increasing proteinuria. The child died at 10 years of age. Two younger siblings of the index case (IV:2 and IV:3, Figure 1A) presented in a similar manner, before 6 months of age, with proteinuria, hypoalbuminaemia and the development of oedema. Their nephrotic syndrome was also resistant to prolonged oral steroid therapy. Long-term follow-up data are unavailable for these siblings.

Results and discussion

We present an index case with infantile NS and two other affected siblings from a consanguineous Saudi Arabian family. Genetic analysis was performed in this family in order to obtain a molecular diagnosis, allowing screening for siblings and for the appropriate long-term management to be undertaken. Genetic analysis was performed in three affected siblings (IV:1, IV:2 and IV:3), one unaffected sibling (IV:4) and both parents (III:1 and III:2) (Figure 1A).

To identify regions of homozygosity and the likely disease locus within this consanguineous family, we carried out a genome-wide linkage search using Affymetrix GeneChipR Human Mapping 10K Arrays (http://www. affymetrix.com). The data were analysed using the Gene Chip DNA Analysis Software 2.0 (GDAS). Two regions of homozygosity across all three affected children were identified using the easyLINKAGE-Plus software (http:// www.uniwuerzburg.de/ nephrologie/molecular_genetics/ molecular_genetics.htm). The first region was 129 Mb in length and was located on chromosome 1 (flanking SNPs: rs692982 and rs1898153). The second region was 11 Mb in length and located on chromosome 17 (flanking SNPs: rs1367950 and rs718994) (Figure 1B). The chromosome 1 region of homozygosity contained the *NPHS2* gene, which was screened by direct sequencing of PCR products of coding regions (8 exons). In exon 3 of the *NPHS2* gene, we found a novel mutation c.385C>T predicted to cause a truncating mutation within podocin (Q129X). The three affected siblings were (as expected) homozygous for the mutation, while both parents were heterozygous for the mutation. The unaffected son was homozygous for the wild-type allele (Figure 1C). In order to detect possible tri-allelism, mutational analysis in other known NS genes was performed. We sequenced *NPHS1* and *PLCE1* and found no mutations. Given that the *NEPH1* gene, whose protein product interacts with podocin, was also located within the region of homozygosity on chromosome 1, this was also directly sequenced. Homozygosity was confirmed within this gene, but no novel variants were identified.

The association of cystic adenomatoid malformation of the lungs in one of the affected children is likely to be a chance association. Extra-renal manifestations with *NPHS2* have occasionally been reported, in the form of cerebral haemangioma and congenital cataract [12]. *NPHS1* mutations have been reported to sometimes give rise to extra-renal manifestations such as microcephaly,

deafness and pulmonary artery stenosis [12]. *LAMB2* mutations are associated with Pierson syndrome [13].

This homozygous truncating mutation Q129X within *NPHS2* is novel. The wild-type podocin protein is 383 amino acids in length and is predicted to have prohibitin homologues (PHB) protein domains between amino acids at position 123–282. The truncation at amino acid position 129 truncates the protein within this domain (Figure 1D) and would be predicted to lead to loss of function. A previously reported *NPHS2* homozygous mutation nt(465/6) ins T in exon 4, leading to a predicted stop codon at amino acid position 166, resulted in severe CNS [1]. In addition, a recent report from Turkey documented a homozygous Q39fsX60 mutation in a child with sporadic NS [6]. The severity of the reported Q129X mutation in *NPHS2* correlates with the early-onset SRNS and the poor outcome in this family and is consistent with previous reports where nonsense, frameshift and homozygous R138Q mutations result in early-onset SRNS [14].

The detection of an *NPHS2* mutation in CNS or infantile NS has implications for treatment. Indeed, given the fact that 85% of NS developing in the three first months of life and 66% of NS occurring within the first year of life are caused by mutations in one of the four genes *NPHS1*, *NPHS2*, *WT1* and *LAMB2* [5], the use of steroids is not recommended in patients with NS of early onset.

Treatment regimes for nephrotic syndrome must be directed towards limiting proteinuria. Both prednisolone and calcineurin inhibitors have been widely used, based on the assumption that nephrotic syndromes were immune-mediated diseases. Interestingly, cyclosporin, as well as its immunosuppressive effects, has recently been shown to stabilize the actin cytoskeleton within podocytes. The proposed mechanism is a reduction in the calcineurin-mediated dephosphorylation of synaptopodin. The abundance of synaptopodin maintains the integrity of the glomerular barrier and stabilizes the antiproteinuric function of the glomerular basement membrane [15]. There is also evidence that corticosteroids may play a role in actin stabilization [16]. The utility of these treatments is not proven in patients with podocin mutations, but remission rates using steroid and cyclosporin in Japanese children with steroid-resistant NS were >80% [17].

Homozygous or compound heterozygous *NPHS2* mutations predict a greater risk of progression to ESRF [6]. If renal transplantation had been possible in the affected individuals, then recurrence risk of steroid-resistant NS would be predicted to be low, although not totally excluded [4,18]. The most common renal histological lesion associated with *NPHS2* mutations is FSGS, typically seen in 80% of cases of NS associated with *NPHS2* mutations [18]. The incidence of mesangioproliferative glomerulonephritis is reported in ~2–4% of cases of SRNS with proven *NPHS2* mutations [18]. Interestingly, reports of another severe truncating mutation (R138X) in children from an Israeli-Arab kindred also describe histological findings of mesangial proliferation [19]. Unfortunately, the disease spectrum of this novel podocin mutation (Q129X) is not known and cannot be predicted by a single family. Identical mutations may have different phenotype depending on certain modifying factors [14].

We suggest that in single affected cases of congenital and childhood nephrotic syndrome, sequencing of the most common implicated genes *NPHS1*, *NPHS2*, *WT1* and *LAMB2* should be performed in the first instance. Previous reports in European and Turkish children detected mutations in two-thirds of cases by sequencing these genes [5]. In cases of multiplex families with consanguinity, such as this case, genome-wide SNP analysis looking for regions of homozygosity remains an effective method for locating loci where mutations may lie. In the future, a chip array allowing genotyping of multiple podocyte-expressed genes, perhaps combined with tissue expression patterns from renal biopsies, may allow better classification of inherited nephrotic syndromes. The need to screen multiple podocyte-related genes is emphasized by the oligogenic/digenic inheritance of CNS and SRNS. Tri-allelic involvement of *NPHS1* and *NPHS2* leading to CNS has previously been reported [1,20]. Weber *et al*. describe a child with SRNS at birth in whom a combination of a heterozygous *de novo* splice mutation in *NPHS1* and a homozygous *NPHS2* R138Q mutation was detected [4]. To our knowledge, the association of a nonsense mutation in *NPHS2* with a heterozygous change in *NPHS1* or other implicated gene has not been reported.

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