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Novel *NPHS1* splice site mutations in a Chinese child with congenital nephrotic syndrome

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ABSTRACT. Congenital nephrotic syndrome (CNS) is defined as heavy proteinuria or nephrotic syndrome occurring before 3 months of age. It is characterized by early onset and progresses to end-stage renal disease. Recently, several genes associated with CNS have been identified, including *NPHS1* and *NPHS2*. Mutations in the *NPHS1* gene have been identified in patients with CNS in Finland with relatively high frequency. Thus far, only a few case reports about CNS have described an *NPHS1* mutation in China. In this study, mutational analyses of *NPHS1* and *NPHS2* were performed in a Chinese child with CNS. Mutations were analyzed in all exons and exon/intron boundaries of *NPHS1* and *NPHS2* in the patient and his parents as well as in 50 unrelated controls using polymerase chain reaction and

direct sequencing techniques. No mutations were detected in *NPHS2*. A novel splice site mutation (IVS11+1G>A) within intron 11 and a missense mutation within exon 8 (c.928G>A) in the *NPHS1* gene were detected in the child. The child's mother had normal urinalysis and a c.928G>A (D310N) heterozygous mutation, and his father had normal urinalysis and IVS11+1G>A. These were not identified in the 50 unrelated controls. The novel splice site mutation of IVS11+1G>A and a missense mutation at c.928G>A in *NPHS1* were found to cause CNS in this Chinese child.

Key words: Chinese; Congenital nephrotic syndrome; *NPHS1*

INTRODUCTION

NPHS1 is localized on chromosome 19q13.1 and codes for the nephrin protein, an essential component of the interpodocyte-spanning slit diaphragm (SD) (Kestilä et al., 1998). Nephrin is a transmembrane protein of the immunoglobulin (Ig) superfamily; it contains an extracellular domain with 8 IgG-like motifs and a fibronectin type-like motif, a transmembrane domain, and a cytosolic C-terminal end (Kestilä et al., 1998). Nephrin forms a zipper-like filter structure in the center of the slit and plays an important role in cell-cell signaling in the SD (Pätäri-Sampo et al., 2006).

The *NPHS1* gene has been identified as the causative gene of congenital nephrotic syndrome (CNS) of the Finnish type (CNF), a rare autosomal recessive disorder. This disease is characterized by massive proteinuria, a large placenta, and the onset of nephrotic syndrome within the first 3 months of the life. The course of CNF is progressive, often leading to end-stage renal disease within 2-3 years of age (Kestilä et al., 1998; Heeringa et al., 2008).

Mutations in the *NPHS1* gene lead to disruption of the filtration barrier and cause massive protein loss. To date, more than 160 different mutations, including deletions, insertions, nonsense, missense, splice site, and promoter mutations, have been reported both in Finnish and non-Finnish patients (Lenkkeri et al., 1999; Beltcheva et al., 2001; Yu et al., 2012; Ameli et al., 2013).

NPHS1 is relatively common in the Finnish population, with an incidence of 1:8200 newborns (Ahvenainen et al., 1956; Norio, 1966). This frequency is much lower in other countries (Fuchshuber et al., 1996). The mutation detection rate of *NPHS1* approaches 98% in Finnish cases. However, *NPHS1* genetic screening in patients of non-Finnish origin has shown a detection rate between 39-80% (Lenkkeri et al., 1999; Hinkes et al., 2007; Heeringa et al., 2008; Ismaili et al., 2009; Schoeb et al., 2010). Only a few cases regarding *NPHS1* mutations in CNS have been reported in China (Shi et al., 2005; Wu et al., 2011; Yu et al., 2012). In this study, we identified novel *NPHS1* splice site mutations in a Chinese child with CNS.

MATERIAL AND METHODS

Patients and data recruitment

A Chinese boy was admitted to the Department of Pediatrics, Puyang Youtian Hospital at 70 days of age for evaluation of edema, which occurred 25 days after birth. He was a

full-term normal delivery baby with a birth weight of 2.5 kg. The weight of the placenta was unknown. Urinalysis showed 3+ protein and microscopic hematuria (4-10 erythrocytes per high-power field). Serum albumin was 16.9 g/L and serum cholesterol was 5.62 mM. There was no evidence of congenital infection. Screening of blood serum and urine from the patient excluded the presence of antibodies for toxoplasma, rubella virus, cytomegalovirus, herpes simplex virus, and organic acid metabolic disorder. Renal biopsy was refused by his parents. The patient was diagnosed with CNS. Both the father and the mother were 24 years old and showed normal urinalysis results. Parental consanguinity was denied. The other members of the family had no history of renal disease.

We performed mutational analysis of *NPHS1* encoding nephrin and *NPHS2* encoding podocin, the most frequent monogenic causes of childhood CNS. This study was approved by the Ethics Committee of the Hospital of Puyang.

Mutation analysis

Polymerase chain reaction (PCR) amplification of genomic DNA was conducted after obtaining informed consent. Peripheral blood samples from the boy and his parents as well as from 50 controls were obtained for genetic analysis in tubes containing potassium oxalate. Genomic DNA was isolated from blood samples using an E.Z.N.A Blood DNA purification kit (OMEGA Bio-Tek, Norcross, GA, USA) following manufacturer guidelines. Mutation analysis of *NPHS1* and *NPHS2* was performed using PCR and direct sequencing. Primers were designed to cover the sequences of all exons and introns adjacent to each exon of *NPHS1* and *NPHS2*, based on published primer sequences (Lenkkeri et al., 1999; Tsukaguchi et al., 2002). PCR products were directly sequenced using the ABI PRISM Big Dye kit using the same amplification primers with an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Sequences were analyzed using the DNAStar software. For references to *NPHS1* and *NPHS2*, the published wild-type sequences (NM_004646 and NM_014625) were used for both nucleotide and amino acid numbering. For all detected mutations and variants, both strands were sequenced. Whenever possible, segregation was confirmed by direct sequencing of the parental samples.

Urinalysis of the 50 control subjects was normal. Novel mutations of *NPHS1* were investigated in the 50 healthy controls by direct sequencing

RESULTS

No mutation in *NPHS2* was detected. Two heterozygous single-base mutations (c.928G>A and IVS11+1G>A) in *NPHS1* were identified in the child with CNS. The c.928G>A mutation within exon 8 has been published previously (Shi et al., 2005) and can cause an amino acid substitution (D310N). A novel splice site mutation IVS11+1G>A (c1440+1G>A) within intron 11 has not been previously reported; this mutation may cause a truncated nephrin molecule lacking the intracellular and transmembrane domains, whereas this was not observed in the 50 controls. Further mutational analysis of the *NPHS1* gene of the parents showed a heterozygous mutation, IVS11+1G>A, in the father (Figure 1) and a heterozygous mutation, c.928G>A, in his mother.

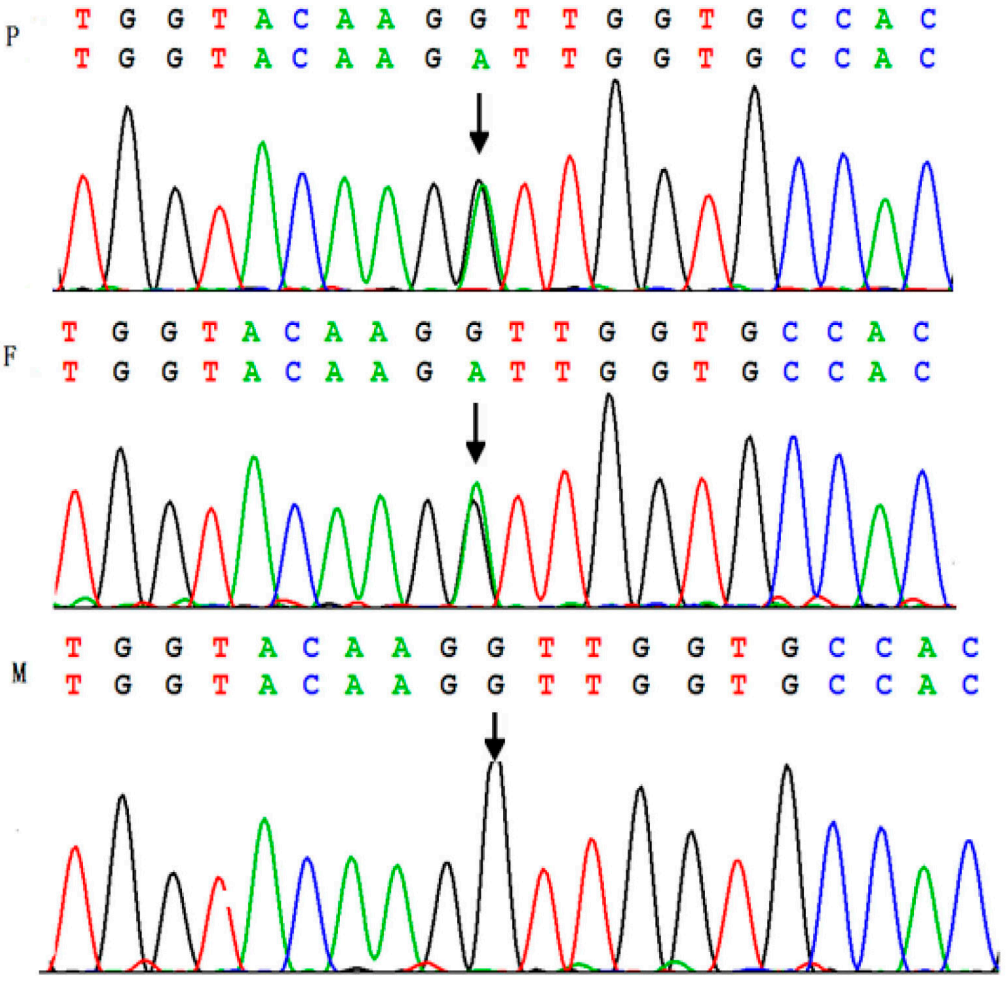

Figure 1. Novel splice site mutations IVS11+1G>A of the *NPHS1* gene were identified by sequencing a Chinese child with congenital nephrotic syndrome. Chromatogram by sequencing of exon 11 of *NPHS1* from the patient (P), father (F), and mother (M). The arrows indicate mutant positions.

DISCUSSION

We found that the Chinese boy with CNS carried 2 heterozygous mutations in *NPHS1*, a splice-site mutation (IVS11+1G>A) and a missense mutation (c.928G>A). The splice site mutation (IVS11+1G>A) is predicted to abrogate splice acceptor function and lead to abnormal splicing of exon 11. This mutation is located in Ig5 and may result in a truncated nephrin molecule lacking intracellular and transmembrane domains (Lenkkeri et al., 1999). This indicates that this previously undescribed mutation is indeed a pathogenic mutation. The missense mutation (c.928G>A), which is predicted to result in a 310-aspartic acid that is replaced with asparagine glutaminea in Ig3, results in misfolding and defective intracellular transport and the

consequent absence of the mutant nephrin on the plasma membrane (Liu et al., 2001). This mutation has been previously reported and accepted as a pathological mutation (Shi et al., 2005).

NPHS2 gene mutations have also been identified as causative genes in CNS patients (Jalanko, 2009). However, we analyzed all 8 possible mutations in *NPHS2*, and found no causative mutations in *NPHS2* in this child.

Based on our analysis, these two heterozygous mutations in the *NPHS1* gene were deemed to be disease-causing mutations in this patient.

The *NPHS1* gene consists of 29 exons and has a genomic size of 26 kb, with a coding region of 4.3 kb. The nephrin protein is composed of 1241 amino acids and is homologous to cell adhesion molecules of the Ig superfamily. Several cell adhesion molecules have been shown to have diverse functions in cell-cell or cell-matrix interactions. These molecules have an extracellular domain of 8 Ig-like modules, a fibronectin type III-like module, a single transmembrane domain, and a cytosolic C-terminal end (Kestilä et al., 1998). Nephrin plays a significant role in promoting cellular contacts through direct nephrin-nephrin interactions through the contribution of other SD components (Khoshnoodi et al., 2003). Phosphorylated nephrin binds to Nck, which is an adapter protein that is involved in reorganizing the cell's actin cytoskeleton in podocytes (Jones et al., 2006).

In 1998, mutations in *NPHS1* (AF035835) were first reported to be associated with CNS by Kestilä et al. (1998). More than 160 different mutations in *NPHS1*, mostly in exons, have been published (Schoeb et al., 2010), of which 2 types of classical mutations are most commonly observed in Finnish patients, including Finmajor (nt121delCT, L41fsX91) in exon 2 and Finminor (c.3325C>T, R1109X) in exon 26. The *NPHS1* mutation detection rate approaches 98% in children with CNS in Finland (Kestilä et al., 1998). However, outside of Finland, the *NPHS1* mutation detection rate is 39-80% in cases with CNS (Lenkkeri et al., 1999; Hinkes et al., 2007; Heeringa et al., 2008; Ismaili et al., 2009; Schoeb et al., 2010). Currently, only 8 *NPHS1* mutations have been described in Chinese individuals, including 1 deletion (c.1983-1900del8) (Shi et al., 2005), 1 insertion (c.3250insG) (Yu et al., 2012), 1 nonsense mutation (c.2783C>A) (Wu et al., 2011), and 5 missense mutations (D310N, Q453R, I742T, R800C, V957L) (Shi et al., 2005; Mao et al., 2007; Wu et al., 2011). In this study, we found a novel splice-site mutation (IVS11+1G>A) in *NPHS1* that caused CNS in a Chinese child. Information regarding the frequency and ethnic distribution of known mutations will help us to understand the pathogenesis of CNS.

We identified 2 heterozygous mutations, c.928G>A (D310N) in exon 8 and IVS11+1G>A in intron 11, of the *NPHS1* gene in the Chinese patient, demonstrating that *NPHS1* mutation can cause Chinese CNS. Further mutational analysis of the *NPHS1* gene in the parents of the patient revealed that the 2 heterozygous mutations, c.928G>A and IVS11+1G>A, were of paternal and maternal origin. Therefore, we provided genetic counseling and prenatal diagnosis for the family, as they appeared to be at high risk.

We also detected 5 variants (c.349G>A (rs3814995), c.3315G>A (rs2071327), IVS8+68A>G, IVS24+36C>T, and IVS27+45C>T) of *NPHS1* in the patient, his parents, and some controls, which are thought to be *NPHS1* polymorphisms. Of these, c.349G>A, which causes a glutamic acid to lysine substitution (E117K), has been identified previously in Europeans and Chinese patients (Lenkkeri et al., 1999; Shi et al., 2005). The common polymorphism, c.3315G>A, which does not result in an amino acid substitution, has previously been found in Finnish and Chinese patients (Lahdenkari et al., 2004; Shi et al., 2005). The polymorphism IVS8+68A>G has previously been found in Chinese patients (Shi et al., 2005), and

IVS24+36C>T and IVS27+45C>T have been published in the SNP database of the National Center for Biotechnology in the United States.

Previously, there were only 3 articles describing cases of children with CNS that detected *NPHS1* gene mutations in China. Our study expands the number of novel mutations in *NPHS1*. In conclusion, molecular analysis shows that *NPHS1* mutations are an important cause of CNS in China.

Although CNS is uncommon in China, screening for mutations in the *NPHS1* gene supports definitive diagnosis, which may be useful for clinical management of such patients as well as family counseling and family planning.

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