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Mutations in *NPHS1* in a Chinese child with congenital nephrotic syndrome

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ABSTRACT. Since the identification of the *NPHS1* gene, which encodes nephrin, various investigators have demonstrated that the *NPHS1* mutation is a frequent cause of congenital nephrotic syndrome (CNS); it is found in 98% of Finnish children with this syndrome and in 39-80% of non-Finnish cases. In China, compound heterozygous mutations in the *NPHS1* gene have been identified in two Chinese families with CNS. To our knowledge, however, whether or not *NPHS1* is the causative gene in sporadic Chinese CNS cases has not been established. We identified a homozygous mutation of *NPHS1*, 3250insG (V1084fsX1095), in a Chinese child with sporadic CNS. This finding leads us to suggest that *NPHS1* mutations are also present in sporadic Chinese CNS cases. This gives additional support for the necessity for genetic examination of mutations in the *NPHS1* gene in Chinese children with sporadic CNS.

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

INTRODUCTION

Congenital nephrotic syndrome (CNS) has been defined as the occurrence of nephrotic syndrome (NS) within the first three months of life. CNS of the Finnish type (CNF), an autosomal recessive disorder, is the most common type of CNS and is characterized by massive proteinuria, which may even start *in utero*, a large placenta, marked edema, and characteristic radial dilatations of the proximal tubules. The course of CNF is progressive, often leading to end-stage renal disease within two to three years of age (Kestilä et al., 1998; Heeringa et al., 2008).

The *NPHS1* gene has been identified as the major gene involved in CNF. The gene for *NPHS1* has been localized to chromosome 19q13.1, which 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 with an extracellular domain with eight 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 SD (Pätäri-Sampo et al., 2006). Mutations of the *NPHS1* gene lead to disruption of the filtration barrier and cause massive protein loss. 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). In China, compound heterozygous mutations of the *NPHS1* gene have been identified in two Chinese families with CNS (Shi et al., 2005; Wu et al., 2011). In this study, we identified a homozygous mutation in the *NPHS1* gene in a Chinese child with sporadic CNS.

MATERIAL AND METHODS

Patient and subjects

A boy, 37 days old, was admitted to the Department of Pediatrics, Fuzhou Dongfang Hospital, for evaluation of edema that occurred six days after birth. He was a full-term normal delivery with a birth weight of 2 kg. The weight of the placenta was unknown. Urinalysis showed 3+ protein. Laboratory tests revealed 22 g/L serum total protein, 4.6 g/L albumin, 5.01 mM cholesterol and 1.3 mM blood urea nitrogen. There was no evidence of congenital infection. Screening of blood serum from the patient excluded the presence of antibodies for toxoplasma, rubella virus, cytomegalovirus, herpes simplex virus, and *Chlamydia trachomatis*. The patient received the standard treatment with corticosteroid at a dose of 4 mg/day and failed to respond to four weeks of prednisone therapy. A renal biopsy was refused by his parents.

His parents had normal urinalysis. The other members of the family had no history of renal disease. We studied, as controls, 50 unrelated adult volunteers whose urinalysis was normal.

Mutational analysis

With the subjects' informed consent, samples of their blood were obtained for genetic analysis in tubes containing sodium oxalate. Genomic DNA was isolated from blood samples using an EZNASE Blood DNA purification kit (OMEGA, USA), and following manufacturer guidelines.

The primers for amplifying exons 1-29 were synthesized according to published information regarding intron-exon boundaries (Lenkkeri et al., 1999). Fifty nanograms of genomic DNA was subjected to 36 cycles of PCR amplification in a 25-µL volume consisting of 1 µL 5 pM sense primer, 1 µL 5 pM antisense primer, 1.5-3.5 mM $MgCl_2$, 100 µM dNTPs, and 1.25-1.5 U Taq polymerase (Promega, USA). DNA was denatured at 94°C for 7 min followed by 36 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 56-64°C, and extension for 1 min at 72°C, and a final extension for 7 min at 72°C (ABI 3730XL, USA). Due to the high GC content of exons 12 and 13, GC buffer I (Takara, Japan) was added to the reaction mixture for amplification.

RESULTS

Mutational analysis was performed by PCR and direct sequencing of all exons of *NPHS1*. A homozygous mutation in exon 24 of *NPHS1*, 3250insG, was identified in the child patient with sporadic CNS (Figure 1), whereas it was not found in the 50 controls. Further mutational analysis of the *NPHS1* gene of the parents of the patient showed a heterozygous mutation, 3250insG, in both the father and mother (Figure 1).

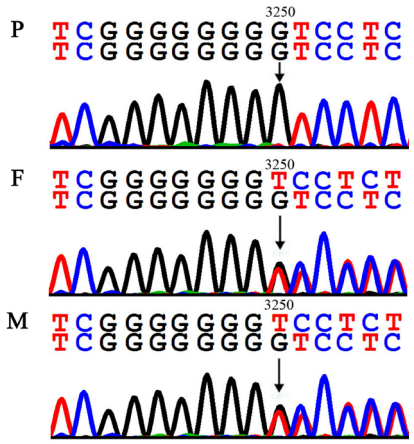

Figure 1. Mutation of the *NPHS1* gene was identified by sequencing Chinese sporadic congenital nephrotic syndrome. Chromatogram by sequencing of exon 24 of *NPHS1* from the patient (**P**), father (**F**), and mother (**M**). The arrows indicate mutant positions.

In addition, we identified three variants, namely 349G>A, 3315G>A, and IVS27+45C>T of *NPHS1*, in the patient, his parents and some controls.

DISCUSSION

We identified a homozygous mutation, 3250insG, in exon 24 of the *NPHS1* gene in the Chinese patient, demonstrating that there is also an *NPHS1* mutation in Chinese sporadic CNS.

The *NPHS1* mutation of 3250insG, which leads to a frameshift and a truncated nephrin protein, V1084fsX1095, was previously reported and accepted as a pathological mutation (Lenk-

keri et al., 1999; Santín et al., 2009; Lee et al., 2009). Santín et al. (2009) considered that nonsense and frameshift mutations, which are predicted to result in a truncated protein, are classified as a severe mutation. In our study, the patient with a homozygous mutation of V1084fsX1095 presented generalized edema at six days after birth and marked hypoalbuminemia, with a serum albumin level of 4.6 g/L, suggesting that severe mutations cause a severe clinical phenotype.

Further mutational analysis of the *NPHS1* gene in the Chinese parents of the patient revealed that the homozygous mutation, 3250insG, was of both paternal and maternal origin. We were therefore able to provide genetic counsel and prenatal diagnosis for the family as they appeared to be at high risk.

We also detected three variants: 349G>A, 3315G>A, and IVS27+45C>T of *NPHS1* in the patient, his parents and some controls, and believe these variants to be *NPHS1* polymorphisms. Of them, 349G>A, which causes a glutamic acid to lysine substitution (E117K), has already been identified in Europeans and Chinese (Lenkkeri et al., 1999; Shi et al., 2005). The common polymorphism, 3315G>A, which does not result in an amino acid substitution, has previously been found in Finnish and Chinese (Lahdenkari et al., 2004; Shi et al., 2005). The polymorphism IVS27+45C>T has been published in the SNP database of the National Center for Biotechnology in the United States.

The *NPHS1* gene mutation detection rate varies among different ethnic groups. The *NPHS1* mutation detection rate approaches 98% in children with CNS in Finland (Kestilä et al., 1998). However, outside 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). Hinkes et al. (2007) reported that 18 patients with CNS in a group of 46 patients from Europe had mutations of the *NPHS1* gene, showing a mutation detection rate of 39%. Ismaili et al. (2009) reported four patients with CNS in a group of 10 patients from Brussels, where the mutation detection rate was 40%. Lenkkeri et al. (1999) reported that 28 patients with CNS in a group of 35 patients from North America, Europe and North Africa were found to have mutations in the *NPHS1* gene, resulting in a mutation detection rate of 80%. Aya et al. (2000) identified a homozygous mutation in the *NPHS1* gene in the proband in Japanese familial CNS. Shi et al. (2005) detected composite heterozygous mutations of the *NPHS1* gene in the proband of a Chinese familial CNS. Wu et al. (2011) have recently identified two novel *NPHS1* mutations in the proband of another Chinese familial CNS.

Although the *NPHS1* gene is the main causative gene that has been identified in patients with CNS, other genes, including *NPHS2*, *WT1*, *LAMB2*, and *PLCE1* have also been identified as causative genes in CNS patients (Jalanko, 2009). Hinkes et al. (2007) reported that the distribution of these genes is *NPHS1* 39.1%, *NPHS2* 39.1%, *WT1* 2.2%, and *LAMB2* 4.4%. These observations suggest that for patients presenting CNS the *NPHS1* gene should be examined first, with mutational analysis of the *NPHS2*, *WT1*, *LAMB2*, or *PLCE1* genes being the next step, should mutations in the *NPHS1* gene be excluded. Patients presenting NS in the congenital period accompanied with urogenital abnormality, male pseudohermaphroditism or Wilms tumor should initially be examined for mutational analysis of the *WT1* gene. Patients with CNS accompanied by microcoria or neurological deficits should initially be examined for mutational analysis of the *LAMB2* gene.

The finding of causative mutations in the *NPHS1* gene in this study suggests that this mutation can occur in Chinese patients with sporadic CNS and supports the necessity of genetic testing for mutations in *NPHS1* in Chinese children with sporadic CNS.

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