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Novel NPHS1 Mutations in a Chinese Young Infant with Congenital Nephrotic Syndrome

CH ***Wang***, JH ***Mao***, XL ***Ma***, LP ***Shi***

Abstract ***Background:*** NPHS1 mutation is one of the major causes of congenital nephrotic syndrome (CNS). Studies have confirmed that approximately one-half of CNS cases are caused by recessive mutations in NPHS1. In China, there have been a few reports of NPHS1 mutation in infants with CNS which indicates NPHS1 may be the causative gene in sporadic Chinese CNS. In this study, NPHS1 mutations in a Chinese family with CNS were detected and analysed. ***Methods:*** A five-day-old male infant suffered generalised oedema, proteinuria, hypoproteinaemia, and hypoalbuminaemia. His kidney histology showed characteristics of CNS. Mutation analysis was made of all exons and exon/intron boundaries of NPHS1 in the infant and his parents using polymerase chain reaction and direct DNA sequencing. ***Results:*** Two compound heterozygous mutations, including 1019C>T (P340L) in exon 9 and 3478C>T (R1160Stop) in exon 27, were identified in the infant with CNS. Only 1019C>T (P340L) was identified in mother and 3478C>T (R1160Stop) in father respectively. ***Conclusions:*** These findings reconfirm that NPHS1 gene mutations also present in sporadic Chinese CNS cases. Genetic studies of *NPHS1* gene should be performed in young infant with CNS for genetic counselling.

Key words Congenital nephrotic syndrome; Mutation; *NPHS1* gene

Introduction

Congenital nephrotic syndrome (CNS) is defined as nephrotic syndrome with onset before the 90th day of postnatal life.[1] In CNS, it has been shown that ~85% of the cases are explained by mutations in four genes. The distribution among these four genes is: NPHS1 39.8%, NPHS2 39.8%, WT1 2.2% and LAMB2 4.4%.[2] Many other studies confirm that approximately one-half of CNS cases are caused by recessive mutations in NPHS1.[1]

In China, there have been a few reports of NPHS1 mutation in infants with CNS which indicates NPHS1 may be the causative gene in sporadic Chinese CNS. In our study, we detected two compound heterozygous NPHS1 mutations in a Chinese family with CNS.

Case Report

A 5-day-old boy was admitted to our NICU for evaluation of oedema that occurred three days after birth. He was the first child of two nonconsanguineous healthy parents without any history of renal disease in his family. He was a preterm infant with birth weight of 2.5 kg. His mother had no history of oligohydramnios and the weight

Department of Neonatal Intensive Care Unit, Children's Hospital, Zhejiang University School of Medicine, Hangzhou 310000, China

CH **Wang**（王陳紅） *MD*
XL **Ma**（馬曉路） *PhD*
LP **Shi**（施麗萍） *MD*

Department of Nephrology, Children's Hospital, Zhejiang University School of Medicine, Hangzhou 310000, China

JH **Mao**（毛建華） *PhD*

Correspondence to: Dr JH **Mao**

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of the placenta was unknown. Urinalysis showed 3+ ~ 4+ protein, urine microprotein 3304.5 mg/L, creatinine 1035.6 μmol/L, ratio of urine protein and creatinine 28.21 mg/mg. Laboratory data showed serum total protein of 29.5 g/L, albumin 10.5 g/L, BUN 16.95 mmol/L, creatinine 94.6 μmol/L. Abdominal ultrasound showed increased echogenicity of the bilateral kidney parenchyma with elimination of the corticomedullary border and the sizes were 5.1×2.5 cm^2 and 5.0×2.6 cm^2 on the right and left kidney respectively. Kidney biopsy showed mesengial proliferation in part of glomeruli, stenosis of capillary lumen, swelling of tubular cells and expansion in part of proximal tubule lumen, suggestive for CNS (Figures 1a &1b). Congenital infections were excluded. TORCH, syphilis studies were negative and immunologic investigations were normal. Thyroid function tests showed hypothyroidism (TSH: 10.59 mIU/L, FT3: 1.7 pmol/L, FT4: 7.0 pmol/L). Echocardiography showed atrial septal defect (ASD). No other abnormality was found.

The patient was on ventilation because of pulmonary haemorrhage on the 2nd day of life. He received several administrations of albumin, antibiotics for pneumonia form 7th day of life, and other supportive treatment, but the condition didn't improved. He died at the age of 20 days after medical care withdrawal.

Genetic analysis was conducted after obtaining informed consent. Genomic DNA samples of the patient and his parents were extracted from peripheral blood by salting out method, according to standard procedures. Mutation analysis of NPHS1 was performed using polymerase chain reaction and direct DNA sequencing. Primers were designed to cover the sequences of all exons and introns adjacent to each exon of NPHS1, according to published primer sequences. Sequences were analysed with the Variant Reporter software and compared to the reference sequences deposited in the public database (NCBI).

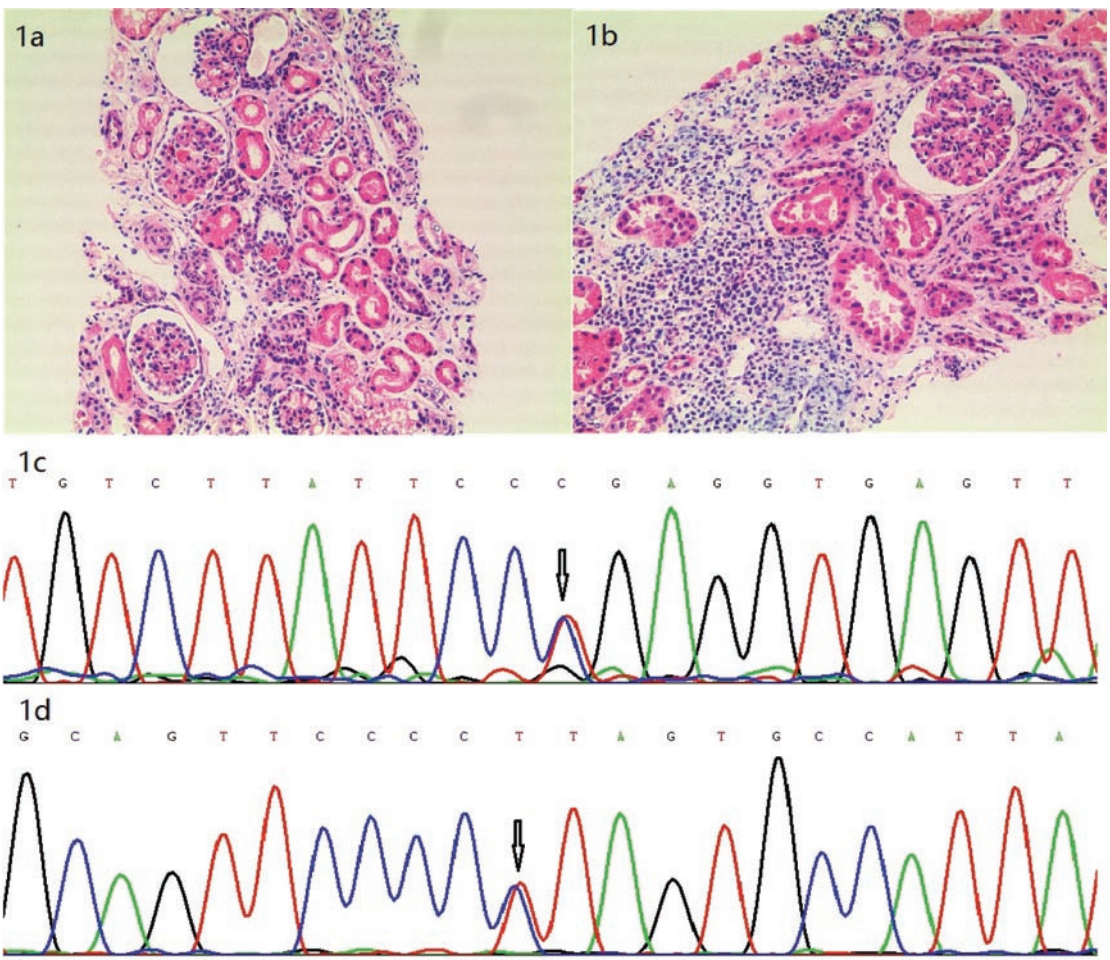

Figure 1 (a, b) Kidney biopsy of the infant. (c) Mutation 1019C>T(P340L) (exon 9) in both the infant and mother. (d) Mutation 3478C>T (R1160Stop) (exon 27) in both the infant and father.

Results

Two compound heterozygous mutations, including 1019C>T (P340L) in exon 9 (Figure 1c) and 3478C>T (R1160Stop) in exon 27 (Figure 1d), were identified in the infant with CNS. Only 1019C>T (P340L) was identified in mother and 3478C>T (R1160Stop) in father respectively. In addition, we identified one variant, namely 349G>A, in both the infant and his parents.

Discussion

Congenital nephrotic syndrome is mainly caused by genetic defects in the components of the glomerular filtration barrier, especially nephrin and podocin. CNS may also be a part of a more generalised syndrome or caused by a perinatal infection (Table 1).[3]

NPHS1 mutation was first diagnosed in Finland where its prevalence is particularly high due to a founder mutation effect.[4] The two main mutations in Finnish population are Fin major (L41fsX90) and Fin minor (R1109X), both leading to the absence of nephrin at the slit diaphragm structure.[4] Outside Finland, however, this Finnish-type CNS is less frequent, and a diversity of different mutations are found in NPHS1,[5] including deletions, insertions, nonsense, missense, splice site and promoter mutations. Hinkes et al[2] reported that 18 patients in a group of 46 patients with CNS from Europe had mutations of the *NPHS1* gene. Schoeb et al found bi-allelic mutations in 36 of the 62 families (58%) in a worldwide cohort confirming that about one-half of CNS is caused by NPHS1 mutations.[1] Up to date, there have been only a few reports of NPHS1 mutation in infants with CNS in China. Shi et al[6] detected composite heterozygous mutations of the NPHS1 gene in the proband of a Chinese familial CNS, including G928A (D310N), 1893-1900del 8 (CGAAACCG), and G2869C (V957L). Wu et al[7] identified a heterozygous nonsense mutation (c.2783C>A) and a missense mutation (c.2225T>C). Recently, Yu et al[8] also identified a homozygous mutation, 3250insG (V1084fsX1095) in the proband of another Chinese familial CNS.

Table 1 The aetiology of congenital nephrotic syndrome

Primary CNS
Nephrin gene mutations [NPHS1, Finnish type of CNS (CNF)]
Podocin gene mutations (NPHS2)
WT1 gene mutations (Denys-Drash, isolated CNS)
LamB2 gene mutations (Pierson syndrome, isolated CNS)
PLCE1 gene mutations
LMX1B mutations (nail-patella syndrome)
LamB3 gene mutations (Herlitz junctional epidermolysis bullosa)
Mitochondrial myopathies
CNS with or without brain and other malformations (no gene defect identified as yet)
Secondary CNS
Congenital syphilis
Toxoplasmosis, malaria
Cytomegalovirus, rubella, hepatitis B, HIV
Maternal systemic lupus erythematosus
Neonatal autoantibodies against neutral endopeptidase
Maternal steroid-chlorpheniramine treatment

The protein nephrin, which contains eight immunoglobulinlike domains, a fibronectin type III-like domain, a transmembranous domain and a short intracellular domain, is coded by NPHS1 gene.[4] Nephrin represents an important structural and signaling protein in podocytes, that regulate ultrafiltration of proteins at the slit diaphragm and connects foot processes in a zipper-like fashion. Mutations of the NPHS1 gene lead to disruption of the filtration barrier and cause massive protein loss.[2,9] Santín et al[9] considered that nonsense and frameshift mutations, which are predicted to result in a truncated protein, are classified as severe mutations. In our study, the NPHS1 mutation of 3478C>T (R1160Stop) within exon 27, which hasn't been previously reported, causes a premature termination of translation, creating a truncated protein of 1160 amino acids. This truncated nephrin has lost the intracellular domains, which can seriously affect the function of nephrin protein. While the previously unpublished single-base mutation of 1019C>T within exon 9 causes an amino acid substitution (P340L), which happens in the immunoglobulin (Ig) domains of nephrin, thus affect the function of nephrin. According to the results, the patient's mutation of 1019C>T (P340L) was from his mother and 3478C>T (R1160Stop) was from his father. Both parents' phenotypes were normal, thus these two compound heterozygous mutations were speculated to be pathogenic to the severe phenotype of the patient.

Diversity of NPHS1 mutation may be the major cause of the heterogeneity of phenotype. Cil et al[10] studied the genotype-phenotype correlations and prognosis in patients with CNS and INS. They found that female patients with

NPHS1 mutations, and NPHS1 mutations affecting the transmembrane or intracellular domains of nephrin were associated with longer survival.

Our study reconfirms that *NPHS1* gene mutations also present in sporadic Chinese CNS cases. Genetic studies of *NPHS1* gene should be performed in infant with CNS for clinical management of such patients, as well as family genetic counselling.

Declaration of Interest

None to declare.

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