RESEARCH NOTE

# Novel *NPHS1* gene mutations in a Chinese family with congenital nephrotic syndrome

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## Introduction

Congenital nephrotic syndrome (CNS) of the Finnish type (CNF; MIM: 256300) is a rare autosomal recessively inherited disease characterized by a large placenta, massive proteinuria, marked edema and clinical onset within the first three months of life. Mutations of *NPHS1* gene were the most well-recognized causes of CNF, which was firstly and mostly described in Finland with two main defects: Finmajor (c.121delCT) and Finminor (c.3325C>T). In other countries, the two typical Finnish mutations were rarely found, but a wide variety of different mutations have been described (Heeringa *et al.* 2008; Schoeb *et al.* 2010), including missense, nonsense, deletions, insertions and splice site. To date, about 183 different *NPHS1* mutations in CNF have been reported, but only a few have been reported in China (Shi *et al.* 2005; Wu *et al.* 2011; Yu *et al.* 2012). In this study, we report a Chinese family with two CNF patients and identified two novel heterozygous missense mutations in exons 15 and 16 of *NPHS1* gene.

## Materials and methods

### *Patients*

Two siblings were diagnosed with CNF, who came from a Chinese nonconsanguineous family with no known Finnish ancestry (figure 1). The proband (III-2), a 15-day-old girl, was admitted to our hospital due to leg oedema, heavy proteinuria (+++) and hematuria (+++). Informed consent was obtained from her parents. The study obtained the ethic committee approval from the ethic commission of Huazhong University of Science and Technology.

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### *Mutations of NPHS1 gene*

Genomic DNA of the proband and her parents was extracted directly from peripheral blood leukocytes using QIAamp DNA Mini kit (Qiagen, Valencia, USA). The primer for amplifying exons 1–29 of *NPHS1* were designed to cover all exons and introns adjacent to each exon of *NPHS1*, according to published primer sequence (Lenkkeri *et al.* 1999). PCR was performed on C1000 Thermo cycler (Biorad, Berkeley, USA) with 2× PCR mix (Thermo Scientific, Waltham, USA). PCR products were identified by agarose gel electrophoresis and purified. Purified PCR products sequencing reaction was performed on an ABI 3730 sequencing reaction with the ABI Big Dye terminator cycle sequencing kit (ABI, Foster City, USA). Sequencing results were analysed using the vector NTI 11. The mutations were described according to the standard nomenclature (den Dunnen and Antonarakis 2000).

To identify the novel mutations, PCR and sequencing of exons 15–16 were performed on 150 unrelated healthy individuals who had the same ethnic background as the patient. The possible impact of the amino acid substitution on the function and structure of nephrin was predicted by the Polyphen-2 software (Adzhubei *et al.* 2010) (http://genetics.bwh.harvard.edu/pph2/).

## Results

### *Clinical features*

The proband (figure 1, III-2) with normal female external manifestations was born at 38 weeks of gestational age and weighed 3500 g at birth. There was no abnormality on routine prenatal examinations. It was recorded that the placenta was twice as large as normal size. The proband was admitted to our hospital due to leg oedema and heavy proteinuria (+++) on the 15th day of birth. An initial physical examination

**Keywords.** congenital nephrotic syndrome of Finnish type; compound heterozygous mutations; *NPHS1* gene; Chinese family.

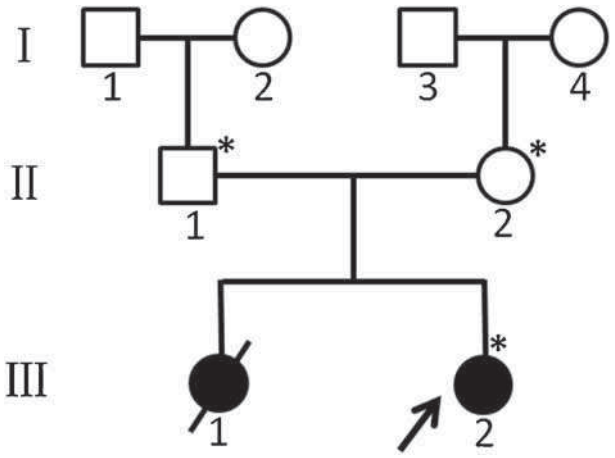


**Figure 1.** The Chinese family pedigree. The arrow indicates the proband and the asterisks indicate blood sample collected.

revealed apparent lower limbs oedema. Initial laboratory test results are shown in table 1. Her elder sister (figure 1, III-1) was also diagnosed with CNS and died of uremia.

***Molecular studies***

Two novel missense mutations of *NPHS1* were found: c.2020C>T and c.2207T>C (figure 2a), both located between Ig-like domain 6 and Ig-like domain 7 of nephrin (figure 2b). The c.2020C>T (p.Pro674Ser) within exon 15 was inherited from father, and was likely to affect the protein structure and function due to the substitution of a hydrophobic amino acid by a hydrophilic amino acid. The c.2207T>C (p.Val736Ala) within exon 16 of *NPHS1* was inherited from mother and its coded amino acid was oriented adjacent to one free cysteine residues. These mutations which had not been published previously were absent in 150 healthy controls and were probably predicted to be damaging with a score of 1.000 (c.2020C>T) and possibly damaging with a score of 0.952 (c.2207T>C) using Polyphen-2. Moreover, the mutation site is highly conserved among humans and other mammals. Therefore, the missense mutations c.2020C>T and c.2207T>C which had not been published previously were speculated to be disease-causing mutations in the Chinese family. Additionally, two other mutations c.349G>A and c.3048+44C>T were also identified in the proband, but these were both single-nucleotide polymorphisms (SNPs) through comparing with SNP database at NCBI and Ensembl.

## Discussion

CNF is a recessively inherited disorder which is most frequent in Finland with the incidence of 1 in 10,000 births. Most CNF patients in Finnish populations (98% of cases) had *NPHS1* mutations (Kestila *et al.* 1998), but CNF was reported outside Finland with a lower frequency of *NPHS1* mutations, accounting for 39–50% of nonFinnish cases (Machuca *et al.* 2010; Schoeb *et al.* 2010). Until now, only six disease-causing mutations are reported in three Chinese CNF patients from three different families. In our study, six Chinese CNF patients were screened for *NPHS1* mutations and only one patient was found to have NPHS1 disease-causing mutations. Together with the total reported *NPHS1* mutations in China, we presumed the frequency of *NPHS1* mutations may be lower in Chinese CNF patients.

In our study, the Chinese patient's disease-causing mutations were compound heterozygous mutations. A recent research showed that 31 of 60 patients (51%) with CNF had compound heterozygous mutations in *NPHS1* (Machuca *et al.* 2010). In China, *NPHS1* mutations in two of three reported CNF patients were compound heterozygous. Thus, compound heterozygous mutations of *NPHS1* gene are very common in CNF patients.

**Table 1.** Laboratory investigation of the proband.

| Laboratory test | Result | Status | Reference value |
|---|---|---|---|
| Albumin | 10 g/L | ↓ | 35–52 g/L |
| Total cholesterol | 6.06 Mm | ↑ | 2.9–5.2 Mm |
| Triglyceride | 1.85 Mm | ↑ | <1.7 Mm |
| LDL | 3.8 Mm | ↑ | 0.0–3.12 Mm |
| IgG | 1.47 g/L | ↓ | 7.00–16.00 g/L |
| IgA | 0.24 g/L | ↓ | 0.7–4.0 g/L |
| Urine protein | 5242.8 mg/L | ↑ | 0.0–150.0 mg/L |
| Urine $\beta$2-microglobulin | 7.29 mg/L | ↑ | 0.0–0.2 mg/L |
| ESR | 43 mm/h | ↑ | 0–20 mm/h |
| APTT | 71.1 s | ↑ | 26.0–38.0 s |
| D-dimer | 2770 ng/mL | ↑ | <500 ng/mL |

LDL, low-density lipoprotein; ESR, erythrocyte sedimentation rate; APTT, activated partial thromboplastin time.

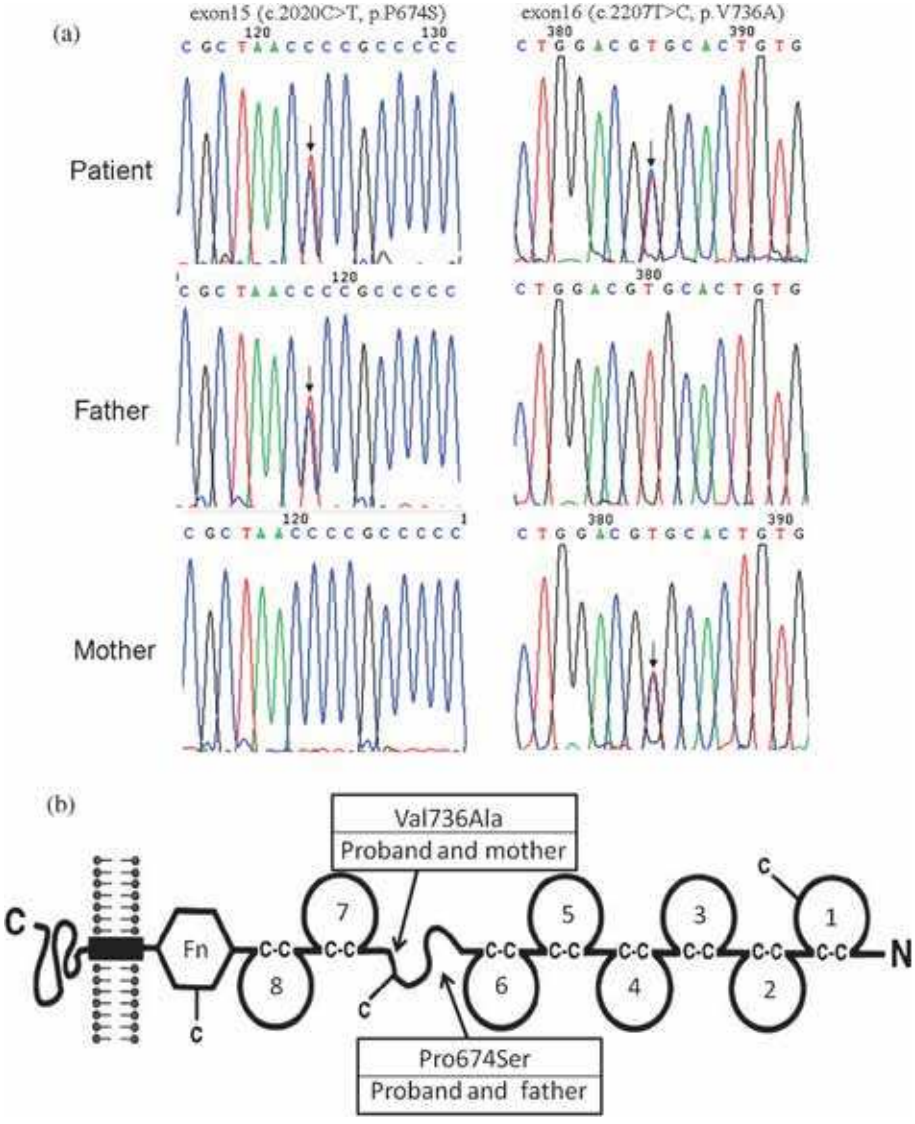


**Figure 2.** Genetic analysis and localization of the two novel mutations in nephrin.

**Table 2.** Pathogenic missense mutations in the *NPHS1* gene detected in cases with CNS from previous studies (Kestila *et al.* 1998; Lenkkeri *et al.* 1999; Aya *et al.* 2000; Patrakka *et al.* 2000; Beltcheva *et al.* 2001; Koziell *et al.* 2002; Caridi *et al.* 2003; Gigante *et al.* 2005; Heeringa *et al.* 2008; Lowik *et al.* 2008; Schoeb *et al.* 2010; Wu *et al.* 2011; Ovunc *et al.* 2012).

| Exon | DNA damage | Effect on protein | Nephrin domain | Origin |
|---|---|---|---|---|
| 2 | c.65C>T | P.Ala22Val | Signal peptide | ND |
| 2 | c.191G>C | p.Trp64Ser | Ig-like domain 1 | Finland |
| 3 | c.313G>A | p.Asp105Asn | Ig-like domain 1 | Japan |
| 3 | c.320C>T | p.Ala107Val | Ig-like domain 1 | India |
| 3 | c.385C>T | p.Leu129Phe | Ig-like domain 1 | Germany |
| 3 | c.386T>A | p.Leu129His | Ig-like domain 1 | Turkey, Armenia |
| 4 | c.479G>C | p.Cys160Ser | Ig-like domain 2 | Japan |
| 4 | c.499C>A | p.Pro167Thr | Ig-like domain 2 | Finland |
| 4 | c.500C>T | p.Pro167Leu | Ig-like domain 2 | India |
| 4 | c.512T>A | p.Ile171Asn | Ig-like domain 2 | Turkey |
| 4 | c.518T>A | p.Ile173Asn | Ig-like domain 2 | France |
| 5 | c.563A>T | p.Asn188Ile | Ig-like domain 2 | India |
| 6 | c.644T>G | p.Leu215Arg | Ig-like domain 2 | Caucasian |
| 6 | c.658T>G | p.Ser220Ala | Ig-like domain 2 | Italy |

**Table 2** (*contd*)

| Exon | DNA damage | Effect on protein | Nephrin domain | Origin |
|---|---|---|---|---|
| 7 | c.767C>T | p.Arg256Trp | Ig-like domain 3 | Arab |
| 7 | c.791C>G | p.Pro264Arg | Ig-like domain 3 | England, India |
| 7 | c.808G>T | p.Gly270Cys | Ig-like domain 3 | England |
| 8 | c.896C>T | p.Arg299Cys | Ig-like domain 3 | France |
| 8 | c.928G>A | p.Asp310Asn | Ig-like domain 3 | China |
| 8 | c.1001T>C | p.Leu334Pro | – | Turkey |
| 9 | c.1019C>A | p.Pro340His | Ig-like domain 4 | Afghanistan, Asia |
| 9 | c.1039G>A | p.Gly347Arg | Ig-like domain 4 | Byelorussia |
| 9 | c.1040G>A | p.Gly347Glu | Ig-like domain 4 | Arab |
| 9 | c.1048T>C | p.Ser350Pro | Ig-like domain 4 | France |
| 9 | c.1096A>C | p.Ser366Arg | Ig-like domain 4 | Croatia, Portugal, North America |
| 9 | c.1099C>T | p.Arg367Cys | Ig-like domain 4 | France, India, Spain |
| 9 | c.1100G>A | p.Arg367His | Ig-like domain 4 | Spain |
| 9 | c.1102C>T | p.Pro368Ser | Ig-like domain 4 | The Netherlands |
| 9 | c.1103C>T | p.Pro368Leu | Ig-like domain 4 | North America |
| 9 | c.1126C>G | p.Leu376Val | Ig-like domain 4 | The Netherlands |
| 9 | c.1135C>T | p.Arg379Trp | Ig-like domain 4 | Japan, Turkey |
| 10 | c.1192T>C | p.Ser398Pro | Ig-like domain 4 | Croatia |
| 10 | c.1219C>T | p.Arg407Trp | Ig-like domain 4 | Jordania |
| 10 | c.1223G>A | p.Arg408Gln | Ig-like domain 4 | Finland, North America |
| 10 | c.1234G>T | p.Gly412Cys | Ig-like domain 4 | Caucasian |
| 10 | c.1250G>T | p.Cys417Phe | Ig-like domain 4 | Turkey |
| 10 | c.1258T>G | p.Phe420Val | Ig-like domain 4 | Germany |
| 11 | c.1337T>A | p.Ile446Asn | Ig-like domain 5 | England, India |
| 11 | c.1339G>A | p.Glu447Lys | Ig-like domain 5 | Japan |
| 11 | c.1379G>A | p.Arg460Gln | Ig-like domain 5 | Mauritania, India, Europe, Spain, Turkey, Antilles, Japan, La Reunion |
| 11 | c.1394G>A | p.Cys465Tyr | Ig-like domain 5 | Finland, France |
| 12 | c.1538T>C | p.Leu513Pro | Ig-like domain 5 | Spain |
| 12 | c.1555C>T | p.Pro519Ser | Ig-like domain 5 | Europe, France |
| 12 | c.1583G>T | p.Cys528Phe | Ig-like domain 5 | France |
| 13 | c.1672C>T | p.Arg558Cys | Ig-like domain 6 | Turkey |
| 13 | c.1707C>G | p.Ser569Arg | Ig-like domain 6 | Jerusalem (Arab) |
| 13 | c.1715G>A | p.Ser572Asn | Ig-like domain 6 | Italy, Algeria |
| 13 | c.1716C>G | p.Ser572Arg | Ig-like domain 6 | Europe |
| 13 | c.1738T>G | p.Trp580Gly | Ig-like domain 6 | Germany |
| 13 | c.1756A>G | p.Arg586Gly | Ig-like domain 6 | Somalia |
| 14 | c.1760T>G | p.Leu587Arg | Ig-like domain 6 | Turkey |
| 14 | c.1801G>C | p.Gly601Arg | Ig-like domain 6 | Japan |
| 14 | c.1829T>A | p.Leu610Gln | Ig-like domain 6 | France |
| 14 | c.1850A>G | p.His617Arg | Ig-like domain 6 | ND |
| 14 | c.1868G>T | p.Cys623Phe | Ig-like domain 6 | North America, England, Spain, Greece |
| 14 | c.1928T>C | p.Leu643Pro | – | India |
| 15 | c.2014G>C | p.Ala672Pro | – | Turkey |
| 15 | c.2014G>A | p.Ala672Thr | – | France |
| 15 | c.2019C>A | p.Asn673Lys | – | Europe |
| 15 | c.2043G>T | c.Trp681Cys | – | Arab |
| 16 | c.2104G>T | p.Gly702Trp | – | France |
| 16 | c.2126T>G | p.Val709Gly | – | Turkey, Portugal |
| 16 | c.2131C>T | p.Arg711Cys | – | Italy |
| 16 | c.2143G>C | p.Gly715Arg | – | Spain |
| 16 | c.2149T>G | p.Tyr717Asp | – | Turkey, Armenia |
| 16 | c.2171C>G | p.Ser724Cys | – | France |
| 16 | c.2175G>C | p.Glu725Asp | – | ND |
| 17 | c.2216C>T | p.Ala739Val | – | ND |
| 17 | c.2225T>C | p.Ile742Thr | Ig-like domain 7 | China |
| 17 | c.2227C>T | p.Arg743Cys | Ig-like domain 7 | Finland, England, France |
| 17 | c.2251G>T | p.Val751Leu | Ig-like domain 7 | France |
| 17 | c.2324G>T | p.Trp775Leu | Ig-like domain 7 | Turkey |
| 18 | c.2404C>T | p.Arg802Trp | Ig-like domain 7 | The Netherlands, India |
| 18 | c.2405G>C | p.Arg802Pro | Ig-like domain 7 | North America |
| 18 | c.2417C>A | p.Ala806Asp | Ig-like domain 7 | Morocco, Spain, France |

**Table 2** (*contd*)

| Exon | DNA damage | Effect on protein | Nephrin domain | Origin |
|---|---|---|---|---|
| 18 | c.2456A>T | p.Asp819Val | Ig-like domain 7 | Japan |
| 18 | c.2491C>T | p.Arg831Cys | Ig-like domain 7 | North America |
| 18 | c.2500G>T | p.Val834Phe | – | France |
| 19 | c.2552C>T | p.Ala851Val | Ig-like domain 8 | ND |
| 19 | c.2587T>C | p.Cys863Arg | Ig-like domain 8 | Morocco |
| 20 | c.2719G>A | p.Ala907Thr | Ig-like domain 8 | Italy |
| 20 | c.2728T>C | p.Ser910Pro | Ig-like domain 8 | Arab |
| 20 | c.2755A>C | p.Thr919Pro | Ig-like domain 8 | Finland |
| 20 | c.2776C>T | p.Leu926Phe | Ig-like domain 8 | Italy |
| 21 | c.2869G>C | p.Val957Leu | FN-III | China |
| 21 | c.2881T>C | p.Trp961Arg | FN-III | France |
| 22 | c.2928G>T | p.Arg976Ser | FN-III | Europe |
| 22 | c.2930A>G | p.Try977Cys | FN-III | Caucasian |
| 24 | c.3230A>G | p.Asn1077Ser | Cytoplasmic | Italy |
| 24 | c.3233C>A | p.Ala1078Asp | Cytoplasmic | Italy |
| 27 | c.3418C>T | p.Arg1140Cys | Cytoplasmic | France |

ND, no data available.

Nephrin is an essential component of the slit diaphragm (SD), the structurally molecular filter in renal glomerular capillaries (Wartiovaara *et al.* 2004). *NPHS1* mutations lead to disruption of the SD and massive protein loss, which has been proved by electron microscopy and electron tomography. To date, the most common *NPHS1* mutations causing CNS are missense mutations, up to about 50% (table 2). Liu *et al.* (2001) showed the pathomechanism of a large number of missense mutations of *NPHS1* gene. They found that nephrin was a highly flexible protein which can lead to misfold and could be retained in the endoplasmic reticulum instead of being transported to the plasma membrane. In this study, the two novel *NPHS1* missense mutations were speculated to affect the protein structure and function. However, the true impact on nephrin function needs to be further tested by experiment *in vitro*.

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