NPHS1 gene mutation in Japanese patients with congenital nephrotic syndrome

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Abstract

Background and Methods. The NPHS1 gene was analysed in different five Japanese patients with congenital nephrotic syndrome (CNS) from the patients in a previous report (Sako M, Nakanishi K, Obana M et al. Analysis of NPHS1, NPHS2, ACTN4, and WT1 in Japanese patients with congenital nephrotic syndrome. Kidney Int 2005; 67: 1248–1255) that suggested that the mutation of NPHS1 was not a major cause of CNS in Japanese patients. Genomic DNA was extracted from leukocytes, and all exons and exon–intron boundaries were analysed for NPHS1 using polymerase chain reaction and direct sequencing.

Results and Conclusions. Compound heterozygous mutations of NPHS1 were found in four patients and homozygous mutations in one patient. Interestingly, three patients out of five had the same mutation in NPHS1: nt2515(delC). Parents who had this mutation heterozygously were from neighbouring prefectures. Two among five patients in this research and one in the previous report (Kidney Int 2005; 67:1248–1255) had the same mutation: 736G > Ti in exon 7. All mutations including these two mutations except for one have never been reported outside of Japan yet.

Keywords: congenital nephrotic syndrome; haplotype; Japanese; nephrin; NPHS1

Introduction

NPHS1 was identified as the causative gene of congenital nephrotic syndrome (CNS) of the Finnish type (CNF), a rare autosomal recessive disorder, characterized by massive proteinuria, a large placenta and the onset of nephrotic syndrome soon after birth, in 1998 [1,2]. Its protein product nephrin is a single pass transmembrane protein consisting of eight extracellular immunoglobulin-like modules, a fibronectin type III-like motif and a cytosolic C-terminal tail, and it is located at the slit diaphragm domain of the podocyte [3]. Since nephrin was identified, many molecules with mutations that lead to proteinuria, such as podocin [4], NEPH1 [5,6], CD2AP [7,8], alpha-actinin-4 [9], TRPC6 [10], Fyn [11], Nck [12] etc., have been discovered. Especially, nephrin/Neph1/podocin are thought to make a complex and form a junction between podocytes that functions as a molecular sieve for glomerular filtration [6,13]. To date, more than 70 different mutations including deletions, insertions, nonsense, missense, splice site and promoter mutations have been reported both in Finnish and non-Finnish patients [14–16]. After we reported homozygous mutations in the NPHS1 gene of Japanese CNS patients in 2000 [17], another Japanese group reported that mutations of NPHS1 was not a major cause of CNS in Japanese patients because only two out of 13 CNS patients had homozygous mutations in the NPHS1 gene, in 2005 [18]. In contrast, two mutations, named Fin-major and Fin-minor, have been found in over 90% of Finnish patients [2] and mutations in both alleles of the NPHS1 gene were found in 11 out of 15 Italian patients [16]. There was also a big difference in the frequency of NPHS2 mutation with familial and sporadic steroid-resistant nephrotic syndrome between the European survey and the Japanese one [19–23]. It is important to clarify the frequency and the kinds of mutations in the NPHS1 gene and other genes of CNS patients in each area. In this research, we examined different five Japanese CNS patients from the patients in the previous report [18].

Methods

Patients

A total of five Japanese CNS patients and their families, different from the previous report [18], were studied after informed consent had been obtained with methods approved by the ethics committees of Okayama University Medical School.

All patients had large placentas (>25% of infant birth weight), the typical clinical finding of onset of proteinuria and nephrotic syndrome.
**Table 1. Clinical information of all patients with CNS**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Placenta weight (&gt;25% of birth weight)</th>
<th>Age at diagnosis of NS</th>
<th>Gender</th>
<th>Renal histological findings of CNF</th>
<th>Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>At birth</td>
<td>F</td>
<td>Yes at 1 mo</td>
<td>Unilateral nephrectomy at 5 mo ESRD at 1 yr 3 mo Transplantation at 5 yr</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>At birth</td>
<td>F</td>
<td>Yes at 1 mo</td>
<td>Unilateral nephrectomy at 5 mo Death due to sepsis at 4 yr 1 mo before ESRD</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>At birth</td>
<td>F</td>
<td>Yes at 3 mo</td>
<td>Unilateral nephrectomy at 4 mo ESRD at 4 yr 4 mo Transplantation at 8 yr</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>At birth</td>
<td>M</td>
<td>No at 3 mo</td>
<td>Unilateral nephrectomy at 3 mo ESRD at 1 yr 5 mo Contralateral nephrectomy at 1 yr 8 mo Transplantation at 4 yr Death due to sepsis at 3 mo</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>1 mo</td>
<td>F</td>
<td>Not performed</td>
<td>ESRD at 1 yr 3 mo</td>
</tr>
</tbody>
</table>

M, male; F, female; mo, month; yr, year; ESRD, end-stage renal disease.
CNF, congenital nephrotic syndrome of the Finnish type.

**Table 2. Mutations in patients with congenital nephrotic syndrome**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nucleotide change</th>
<th>Effect on coding</th>
<th>Grantham score</th>
<th>Exon</th>
<th>Carrier</th>
<th>Mutation status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>479G→C</td>
<td>Cys160Ser</td>
<td>112</td>
<td>4</td>
<td>Father/brother</td>
<td>HET</td>
</tr>
<tr>
<td></td>
<td>736G→T</td>
<td>Glu246stop</td>
<td>7</td>
<td>7</td>
<td>Mother</td>
<td>HET</td>
</tr>
<tr>
<td>2</td>
<td>1135C→T</td>
<td>Arg379Trp</td>
<td>101</td>
<td>9</td>
<td>N/A</td>
<td>HOM</td>
</tr>
<tr>
<td>3</td>
<td>nt2515del(C)</td>
<td>Frameshift</td>
<td>19</td>
<td>19</td>
<td>Mother</td>
<td>HET</td>
</tr>
<tr>
<td></td>
<td>2479C→T</td>
<td>Arg827stop</td>
<td>18</td>
<td>18</td>
<td>N/A</td>
<td>HET</td>
</tr>
<tr>
<td>4</td>
<td>736G→T</td>
<td>Glu246stop</td>
<td>7</td>
<td>7</td>
<td>Father/sister</td>
<td>HET</td>
</tr>
<tr>
<td></td>
<td>nt2515del(C)</td>
<td>Frameshift</td>
<td>19</td>
<td>19</td>
<td>Mother/brother</td>
<td>HET</td>
</tr>
<tr>
<td>5</td>
<td>1801G→C</td>
<td>Gly601Arg</td>
<td>125</td>
<td>14</td>
<td>Mother</td>
<td>HET</td>
</tr>
<tr>
<td></td>
<td>nt2515del(C)</td>
<td>Frameshift</td>
<td>19</td>
<td>19</td>
<td>Father</td>
<td>HET</td>
</tr>
</tbody>
</table>

HET, heterozygous mutation; HOM, homozygous mutation.

Results

**NPHS1 analysis**

Mutational analysis of the NPHS1 gene was carried out in five Japanese patients with CNS (Table 2). In patient 1, a heterozygous missense mutation, Cys160Ser (479G > C in exon 4), and the same heterozygous nonsense mutation, Glu246stop (736G > T in exon 7), as reported in a different Japanese patient with CNS in the previous report, were detected. The first mutation was identified heterozygously in the healthy father and brother and the second mutation was found heterozygously in the healthy mother.

In patient 2, a homozygous missense mutation, Arg160Trp (1135C > T in exon 9), was detected. This mutation has already been reported from Europe [15].

In patient 3, a heterozygous single nucleotide deletion (2515delC in exon 19) and a heterozygous nonsense mutation, Arg827stop (2479C > T in exon 18), inherited from the mother were detected. Though the father could not be analysed, 2515delC seems to be inherited from the father. Both parents are from Saitama prefecture.

In patient 4, the same heterozygous single nucleotide deletion (2515delC in exon 19) as in patient 3, inherited between birth and 1 month of age (Table 1). Renal histological examination had been carried out in four of the five patients. Three of these four patients show histological findings of CNF and one (patient 4) showed diffuse mesangial sclerosis.

DNA sequencing

Genomic DNA was extracted and purified from peripheral leukocytes in whole-blood samples by using a QIAmp DNA blood kit (Qiagen, Hilden, Germany).

Individual exons of NPHS1 were amplified by PCR. The primers for NPHS1 were designed on the basis of previously published information regarding intron–exon boundaries [4,14]. The purified product was cycle-sequenced with Big-Dye terminators (Applied Biosystems, Foster City, CA, USA), and the cycle sequence product was analysed with an automated sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems).

Haplotype analysis

Fluorescence genotyping was performed by using two microsatellite markers (D19S1170 and D19S400). D19S1170 is localized at the 56.69 cM region of chromosome 19 on the Marshfield map and D19S400 at 64.7 cM. Both repeat units consist of four base pairs. NPHS1 exists between these markers. The ABI Prism 310 Genetic Analyzer and gene mapper software were used for detection and analysis.
from the mother who is a native of Saitama prefecture, and the same heterozygous nonsense mutation, Glu246stop (736G>T in exon7), as in patient 1, inherited from the father who is from Shizuoka prefecture were detected.

In patient 5, the same heterozygous single nucleotide deletion (2515delC in exon 19) as in patients 3 and 4, inherited from the father who is from Tochigi prefecture, and a heterozygous missense mutation, Gly601Arg (1801G>C in exon14), inherited from the mother who is a native of Aichi prefecture were detected.

All amino acids substituted by missense mutations that seem to be causative by mode of inheritance were preserved among mouse, rat and human.

The Grantham scale was used to evaluate the biochemical severity of the NPHS1 missense mutations; the results suggested that all missense changes were radical (Table 2) [24]. All missense mutations were absent in more than 100 chromosomes of healthy Japanese controls.

One common polymorphism, Glu117Lys (349G>A), was found in patients 1, 3, 4 and 5, but not in patient 2.

**Haplotype analysis**

Haplotype was constructed assuming a minimal number of recombinations (Table 3).

In patient 5, 184 bp of D19S1170 and 210 bp of D19S400 were inherited from the father with 2515delC. In patient 4, 188 bp of D19S1170 and 210 bp of D19S400 were inherited from the mother with 2515delC. The latter allele was the same as in patient 5. In contrast, the frequency of 210 bp of D19S400 was 1.8% in the healthy Japanese controls. Patient 3 had 184 bp and 192 bp of D19S1170 and 206 bp of D19S400. 184 bp of D19S1170 was the same as in patient 5. The frequency of 184 bp of D19S1170 was 23.1% in the healthy Japanese controls.

Intragenic single nucleotide polymorphism, 3515G>A, was found in the NPHS1 gene of these three patients homozygously. The frequency of the genotype at position 3515 was GG: 1.9%, GA: 58.5% and AA: 39.6% in the healthy Japanese controls.

**Discussion**

This study confirms that mutation in the NPHS1 gene is a significant cause of CNS among Japanese patients too, though a previous paper [18] indicated that the incidence of NPHS1 mutation in Japanese patients with CNS was low. Homozygous or compound heterozygous mutations in the NPHS1 gene were detected in five patients. The same mutation 2515delC in exon 19 was detected in three out of five patients. Three parents with this mutation were from Tochigi prefecture or Saitama prefecture, which are neighbours. Japan is divided into 47 prefectures. Tochigi prefecture is bordered immediately to the north by Saitama prefecture, but Shizuoka and Aichi are far from these prefectures. The findings of haplotype associated with 2515delC support a founder effect in at least two patients to some extent. In addition, two among five patients in this research and one in the previous report [18] had the same mutation: 736G>T in exon 7.

All mutations have, as yet, never been reported outside of Japan except for one. Taking insularity into consideration, these facts suggest that these two mutations may be unique and common among Japanese CNS patients.

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**Conflict of interest statement.** None declared.

**References**


Genome-wide linkage analysis for uric acid in families enriched for hypertension

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Abstract

Background. Uric acid is heritable and associated with hypertension and insulin resistance. We sought to identify genomic regions influencing serum uric acid in families in which two or more siblings had hypertension.

Methods. Uric acid levels and microsatellite markers were assayed in the Genetic Epidemiology Network of Arterio-

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