Detection of the Finnish-Type Congenital Nephrotic Syndrome by Restriction Fragment Length Polymorphism and Dual-Color Oligonucleotide Ligation Assays

Eeva-Liisa Romppanen¹ ² and Ilkka Mononen¹ ³ *

Background: Congenital nephrotic syndrome of Finnish type (NPHS1) is an autosomal recessive disorder characterized by severe proteinuria of intrauterine onset. Ninety-four percent of the Finnish NPHS1 chromosomes have been reported to carry either a 2-bp deletion in exon 2 (FinMajor) or a nonsense mutation in exon 26 (FinMinor) of the NPHS1 gene. The high prevalence of only two mutations in the Finnish population enables the use of molecular techniques in the diagnosis of NPHS1 and for carrier screening.

Methods and Results: We describe two different molecular methods for the detection of the NPHS1 mutations: a PCR-restriction fragment length polymorphism (PCR-RFLP) and a dual-color oligonucleotide ligation assay (OLA). The dual-color OLA, which enables simultaneous detection of the NPHS1 FinMajor and FinMinor mutations, can be used for rapid analysis of large sets of samples. The analysis of 2004 Finnish blood samples revealed 34 carriers of the FinMajor mutation and 1 carrier of the FinMinor mutation, indicating a carrier frequency of 1:59 (95% confidence interval, 1:89–1:44) for the NPHS1 FinMajor mutation and 1:2004 (95% confidence interval, 0 to 1:677) for the NPHS1 FinMinor mutation, respectively.

Conclusion: PCR-RFLP and dual-color OLA are suitable for molecular diagnosis and carrier screening of the major mutations that cause NPHS1.

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4 Nonstandard abbreviations: NPHS1, congenital nephrotic syndrome of Finnish type; RFLP, restriction fragment length polymorphism; OLA, oligonucleotide ligation assay; BSA, bovine serum albumin; and WT, wild-type.
the PCR-mediated generation of an artificial TaqI restriction site in the DNA template containing the Fin\textsubscript{Major} mutation. For the simultaneous detection of the Fin\textsubscript{Major} and Fin\textsubscript{Minor} mutations from a large number of samples, we utilized PCR followed by oligonucleotide ligation assay (PCR-OLA) \( (7, 8) \). Both of the mutation sites were amplified in a single PCR reaction. The reporter probes for the Fin\textsubscript{Major} and Fin\textsubscript{Minor} mutation sites were labeled with different haptens, thus allowing simultaneous detection of two mutation sites in a single microplate well by ELISA. The applicability of the dual-color PCR-OLA for carrier screening of the Fin\textsubscript{Major} and the Fin\textsubscript{Minor} mutations of NPHS1 was tested by analyzing 2004 samples from eastern Finland.

### Materials and Methods

**DNA samples**

Peripheral blood samples \((n = 2004)\) were collected in eastern Finland and made anonymous according to the institutional guidelines. The Split Second\textsuperscript{TM} DNA Preparation Kit (Roche) was used to purify DNA samples from 250 \( \mu \)L of EDTA blood. One DNA sample from a Finnish NPHS1 patient known to be homozygous for the Fin\textsubscript{Major} mutation and one from an individual homozygous for the Fin\textsubscript{Minor} mutation in the NPHS1 gene were kindly provided by Dr. Marjo Kestilä (University of Oulu, Oulu, Finland) and Prof. Karl Tryggvason (Karolinska Institut, Stockholm, Sweden). Genomic DNA was diluted to 50 mg/L with 10 mmol/L Tris-HCl (pH 7.5), 1 mmol/L EDTA.

**Synthesis and modification of oligonucleotides**

The oligonucleotides for the PCR and ligation reactions were synthesized using standard phosphoramidite chemistry. For PCR-RFLP, the target DNA in exon 2 (Fin\textsubscript{Major} site) was amplified using primers \( 5'$-\text{TCTGGGTAATC-3'} \) and \( 5'$-\text{GCCCCCTCCACCACCGTCAG-3'} \), and the target DNA in exon 26 (Fin\textsubscript{Minor} site) was amplified using primers \( 5'$-\text{GGGGCTTGCATAGGGTCACT-3'} \) and \( 5'$-\text{ACCTTCATCTCGGAAAGGTGCA-3'} \). For PCR-OLA, the primer sequences described by Kestilä et al. \((6)\) were used. The nucleotide sequences of the allele-specific and reporter oligonucleotides for the Fin\textsubscript{Major} and Fin\textsubscript{Minor} mutations, and the G3315A polymorphism site are shown in Table 1. For detection of the Fin\textsubscript{Major} mutation in the DNA template containing the Fin\textsubscript{Major} mutation and one from an individual homozygous for the Fin\textsubscript{Minor} mutation site in the DNA template containing the Fin\textsubscript{Major} mutation, 6 nmol of each four deoxynucleotide triphosphates, 0.5 U of DyNAZyme II thermostable DNA polymerase (Finzymes), 10 mmol/L Tris-HCl (pH 8.8 at 25 °C), 1.5 mmol/L MgCl\(_2\), 50 mmol/L KCl, and 1 mL/L Triton-X-100 in a reaction volume of 30 \( \mu \)L. The PCR amplifications were performed in an MJ Research PTC-100 thermal cycler (MJ Research). The cycling protocol included an initial temperature cycle of 95 °C for 5 min, 85 °C for the time needed to add the enzyme, 58 °C for 30 s, and 72 °C for 45 s, followed by a cycle of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. A total of 30 cycles were performed, with final extension at 72 °C for 10 min. For detection of the Fin\textsubscript{Major} mutation by RFLP, 20 \( \mu \)L of a restriction enzyme mixture containing 3 U of TaqI enzyme (Promega), \( 1\times \) Buffer E (6 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 6 mmol/L MgCl\(_2\), and 1 mmol/L dithiothreitol), and 0.1 g/L acetylated bovine serum albumin (BSA) was added directly to the PCR reaction mixture containing the 103-bp PCR product, and the mixture was incubated for 1 h at 65 °C. For detection of Fin\textsubscript{Minor} mutation, 20 \( \mu \)L of restriction enzyme mixture containing 3 U of AvaII enzyme (Promega), \( 1\times \) Buffer C (10 mmol/L Tris-HCl, pH 7.9, 50 mmol/L NaCl, 10 mmol/L MgCl\(_2\), and 1 mmol/L dithiothreitol), and 0.1 g/L acetylated BSA was added to the PCR reaction mixture containing the 239-bp PCR product, and the mixture was incubated for 2 h at 37 °C. After the digestion, the analyses were completed by electrophoresis in a 2.5% agarose gel.

### Table 1. Nucleotide sequences of the OLA oligonucleotides for the detection of the NPHS1 Fin\textsubscript{Major} allele, the Fin\textsubscript{Minor} allele, the A3315 polymorphism, and WT alleles.

<table>
<thead>
<tr>
<th>OLA probe</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2, WT allele</td>
<td>5'$-\text{GCCCTCCACCCGTCAG-3'} $</td>
</tr>
<tr>
<td>Exon 2, Fin\textsubscript{Major} allele</td>
<td>5'$-\text{AGGGGCTTGCATAGGGTCACT-3'} $</td>
</tr>
<tr>
<td>Exon 2, reporter</td>
<td>5'$-\text{GCCCCCTCCACCACCGTCAG-3'} $</td>
</tr>
<tr>
<td>Exon 26, WT allele</td>
<td>5'$-\text{CCTCATATTCGTTCCTGACTCA-3'} $</td>
</tr>
<tr>
<td>Exon 26, Fin\textsubscript{Minor} allele</td>
<td>5'$-\text{CCTCATATTCGTTCCTGACTCA-3'} $</td>
</tr>
<tr>
<td>Exon 26, reporter 1</td>
<td>5'$-\text{GTCCTCTCGACCTTGACAG-3'} $</td>
</tr>
<tr>
<td>Exon 26, reporter 2</td>
<td>5'$-\text{GTCCTCTCGACCTTGACAG-3'} $</td>
</tr>
<tr>
<td>Exon 26, Fin\textsubscript{Minor} allele</td>
<td>5'$-\text{ACCTTCATCTCGGAAAGGTGCA-3'} $</td>
</tr>
<tr>
<td>Exon 26, A3315 polymorphism allele</td>
<td>5'$-\text{AGAGAGGACCGAGTTCAGGAA-3'} $</td>
</tr>
</tbody>
</table>

**PCR-RFLP for detection of the Fin\textsubscript{Minor} and Fin\textsubscript{Major} mutations**

The PCR reaction mixtures contained 25 ng of genomic DNA or 0.5 \( \mu \)L of Split Second DNA sample, 50 ng of the amplification primers for the Fin\textsubscript{Minor} or the Fin\textsubscript{Major} mutation site, 6 nmol of each four deoxynucleotide triphosphates, 0.5 U of DyNAZyme II thermostable DNA polymerase (Finzymes), 10 mmol/L Tris-HCl (pH 8.8 at 25 °C), 1.5 mmol/L MgCl\(_2\), 50 mmol/L KCl, and 1 mL/L Triton-X-100 in a reaction volume of 30 \( \mu \)L. The PCR amplifications were performed in an MJ Research PTC-100 thermal cycler (MJ Research). The cycling protocol included an initial temperature cycle of 95 °C for 5 min, 85 °C for the time needed to add the enzyme, 58 °C for 30 s, and 72 °C for 45 s, followed by a cycle of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. A total of 30 cycles were performed, with final extension at 72 °C for 10 min. For detection of the Fin\textsubscript{Major} mutation by RFLP, 20 \( \mu \)L of a restriction enzyme mixture containing 3 U of TaqI enzyme (Promega), \( 1\times \) Buffer E (6 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 6 mmol/L MgCl\(_2\), and 1 mmol/L dithiothreitol), and 0.1 g/L acetylated bovine serum albumin (BSA) was added directly to the PCR reaction mixture containing the 103-bp PCR product, and the mixture was incubated for 1 h at 65 °C. For detection of Fin\textsubscript{Minor} mutation, 20 \( \mu \)L of restriction enzyme mixture containing 3 U of AvaII enzyme (Promega), \( 1\times \) Buffer C (10 mmol/L Tris-HCl, pH 7.9, 50 mmol/L NaCl, 10 mmol/L MgCl\(_2\), and 1 mmol/L dithiothreitol), and 0.1 g/L acetylated BSA was added to the PCR reaction mixture containing the 239-bp PCR product, and the mixture was incubated for 2 h at 37 °C. After the digestion, the analyses were completed by electrophoresis in a 2.5% agarose gel.

**Dual-color PCR-OLA for detection of the Fin\textsubscript{Minor} and Fin\textsubscript{Major} mutations**

For PCR-OLA, the target DNA in exons 2 and 26 of the NPHS1 gene was simultaneously amplified by PCR. The dual-PCR reaction mixture was similar to the PCR mix-
tured for the PCR-RFLP, but contained the amplification primers for both the Fin\textsubscript{minor} and the Fin\textsubscript{major} mutation sites and 1.0 mmol/L MgCl\textsubscript{2} in a reaction volume of 30 µL. The PCR program consisted of an initial temperature cycle of 95 °C for 5 min, 85 °C for the time needed to add the enzyme, 60 °C for 1 min, and 68 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, 68 °C for 2 min, and a final extension of 68 °C for 8 min.

The PCR mixtures containing the 491-bp (exon 2) and 611-bp (exon 26) PCR products were diluted 1:10 with 1 mL/L Triton X-100. The ligation reactions contained 10 µL of the 1:10-diluted PCR product mixture, 0.15 pmol of each allele-specific and reporter probe, 1.2 U of thermostable ligase (New England Biolabs), and 1× NE Buffer [20 mmol/L Tris-HCl (pH 7.6 at 25 °C), 20 mmol/L potassium acetate, 10 mmol/L magnesium acetate, 10 mmol/L dithiothreitol, 1 mmol/L NAD\textsuperscript{+}, and 1 mL/L Triton X-100] in a total volume of 30 µL. The reaction mixtures were subjected to 10 cycles of 97 °C for 30 s and 63 °C for 2 min, and stopped by the addition of 15 µL of 1 mL/L Triton X-100 containing 0.1 mol/L EDTA. All pipetting steps in the OLA and ELISA were performed using an 8-channel pipette (Biohit) or a Tecan Genesis RSP 150 automated pipetting device (Tecan).

For the assay readout, the reaction mixtures were transferred into the wells of a streptavidin-coated, BSA-blocked microtiter plate, and the biotin-labeled allele-specific oligonucleotides were allowed to bind to the streptavidin for 45 min at room temperature. The PCR products and unligated reporter oligonucleotides were removed from the wells by washing the plate first with 10 mmol/L NaOH containing 0.5 mL/L Tween 20 and then with a washing buffer containing 140 mmol/L NaCl, 0.5 mL/L Tween 20, 10 mmol/L Tris-HCl, pH 7.4. Anti-digoxigenin-alkaline phosphatase (Roche) and anti-fluorescein-horseradish peroxidase (Roche) diluted 1:2500 with the washing buffer containing 10 g/L BSA were added to the wells and incubated 2 h at room temperature. The plate was washed with the washing buffer, and the ELISA amplification system (Life Technologies) was used for detection of the Fin\textsubscript{major} allele. The plate was measured with a Tecan Spectrafluor spectrophotometer (Tecan) at 510 nm. To detect the Fin\textsubscript{minor} allele, the plate was washed, the ABTS substrate (Zymed) was added, and the absorbance was measured at 405 nm. The absorbance of the blank well (no DNA in PCR) was subtracted from the absorbance values of the sample wells. The ratio of the absorbance of the wild-type (WT) allele-specific reaction to the mutant allele-specific reaction was calculated to determine the genotype. DNA samples from a patient homozygous for the Fin\textsubscript{major} allele or the Fin\textsubscript{minor} allele were used as controls in every PCR-OLA series.

**PCR-OLA for Detection of the G3315A Polymorphism**

The DNA region containing the G3315A polymorphism in exon 26 was amplified by PCR using the same conditions as for the RFLP analysis of the Fin\textsubscript{minor} mutation. The ligation reactions containing the WT allele-specific or the polymorphism allele-specific probe and the reporter probe were similar to those used for the dual-OLA reactions. The ligation temperature was 58 °C. After the cycles, ELISA was performed to detect the digoxigenin-labeled reporter probe as described above.

**Results**

The PCR-RFLP analyses for the detection of the NPHS1 Fin\textsubscript{major} and Fin\textsubscript{minor} mutations are shown in Fig. 1. The PCR product of a NPHS1 patient homozygous for the Fin\textsubscript{major} mutation is digested into two fragments of 20 and 83 bp because of the artificial restriction site of TaqI enzyme created by the PCR primer (Fig. 1, lane 1). In the case of a WT genotype, the undigested 103-bp fragment is present (Fig. 1, lane 3) and the carrier of the Fin\textsubscript{major} mutation can be identified by the presence of both the 83- and 103-bp fragments (Fig. 1, lane 2). PCR amplification of DNA from a NPHS1 patient homozygous for the Fin\textsubscript{minor} mutation (Fig. 1, lane 4) and a WT control generated a 239-bp product, which appeared slightly larger on electrophoresis. The undigested DNA fragment can be seen on the gel because of an abolished restriction site of AvaII (Fig. 1, lane 4), whereas for a healthy control, the PCR product is digested into two fragments of 188 and 51 bp (Fig. 1, lane 6). Heterozygous carriers of the Fin\textsubscript{minor} mutation are identified by the presence of all the three fragments (239, 188, and 51 bp; Fig. 1, lane 5).

During the development of PCR-OLA for the Fin\textsubscript{minor} mutation site, the nucleotide sequence analysis revealed a silent mutation (G3315A) located 10 nucleotides upstream from the Fin\textsubscript{minor} mutation in exon 26 of NPHS1. The nucleotide sequences of the antisense strands of the WT and the polymorphic allele are shown in Fig. 2. The prevalence of the polymorphism in the Finnish population was estimated by analyzing 256 blood samples.

![Fig. 1. PCR-RFLP detection of the Fin\textsubscript{major} and Fin\textsubscript{minor} mutations that cause NPHS1.](Image://302x155 to 530x280)

Separation of DNA fragments originating from an amplified 103-bp region of the NPHS1 gene in exon 2 and a 239-bp region in exon 26 after digestion with TaqI and AvaII restriction endonucleases, respectively. For exon 2, a NPHS1 patient homozygous for the Fin\textsubscript{major} allele (lane 1), a carrier of the NPHS1 Fin\textsubscript{major} allele (lane 2), and a WT individual (lane 3) are shown. For exon 26, a NPHS1 patient homozygous for the Fin\textsubscript{minor} allele (lane 4), a carrier of the NPHS1 Fin\textsubscript{minor} allele (lane 5), and a WT individual (lane 6) are shown. Lanes Std, molecular size markers.
Seventy-four of 256 samples showed a WT genotype (95% confidence interval, 60 – 88 of 256), 70 of 256 showed a homozygous genotype for the polymorphism (95% confidence interval, 56 – 84 of 256), and the remaining 112 of 256 samples (95% confidence interval, 96 – 128 of 256) showed a heterozygous genotype. The prevalence of the WT allele (G3315) was 51% (95% confidence interval, 46 – 55%), and the prevalence of the A3315 allele was 49% (95% confidence interval, 45–54%). The result was in overall agreement with the Hardy-Weinberg equilibrium. Because the polymorphism has such a high prevalence in the studied population, it must be taken into consideration when performing PCR-OLA. Although the polymorphism is situated in the middle of the reporter probe (Table 1), it disturbs the ligation of the oligonucleotides, producing a weak or absent signal in ELISA. Therefore two different reporter probes, one specific for the WT sequence (G3315) and the other specific for the polymorphic sequence (A3315), were used in both ligation reactions for the detection of the WT and FinMinor alleles in exon 26.

A schematic overview of the dual-color PCR-OLA for the detection of NPHS1 is illustrated in Fig. 3. The dual-PCR step is followed by two separate ligation reactions (Fig. 3, ligation A for the WT alleles and ligation B for the mutant alleles). The covalent ligation of the oligonucleotides, which occurs in the case of the perfect complementarity between the target DNA and the oligonucleotides, is detected by the color development in ELISA. Seven possible outcomes in dual-color PCR-OLA for the detection of the FinMajor and FinMinor mutations are shown in Fig. 3. A WT individual is positive for reaction A for both of the mutation sites (Fig. 3, outcome 1). A NPHS1 patient homozygous for the FinMajor allele (exon 2, outcome 2) or FinMinor allele (exon 26, outcome 3), a compound heterozygote carrying both of these alleles (exons 2 and 26, outcome 4), a carrier of the FinMajor allele (exon 2, outcome 5) or the FinMinor allele (exon 26, outcome 6), and a negative control or a sample with failure in PCR (exons 2 and 26, outcome 7).

Fig. 3. Principle of dual-color PCR-OLA for the detection of the FinMajor and FinMinor mutations that cause NPHS1.

PCR amplification of the target DNA sequences in exon 2 and exon 26 of the NPHS1 gene is followed by ligation of the biotinylated (B) allele-specific oligonucleotides and the fluorescein (F) or digoxigenin (D)-labeled reporter probes. Ligation A is specific to WT alleles, whereas ligation B is specific to mutant alleles. The biotinylated probes are captured on streptavidin-coated microtiter wells, and the presence of the covalently linked oligonucleotides in the wells is detected using ELISA. Possible outcomes of the assay include a WT individual (outcome 1), a NPHS1 patient homozygous for the FinMajor allele (exon 2, outcome 2) or FinMinor allele (exon 26, outcome 3), a compound heterozygote carrying both of these alleles (exons 2 and 26, outcome 4), a carrier of the FinMajor allele (exon 2, outcome 5) or the FinMinor allele (exon 26, outcome 6), and a negative control or a sample with failure in PCR (exons 2 and 26, outcome 7).
samples homozygous for the WT allele, the absorbance ratio of the A and B reactions was >5, whereas samples homozygous for the mutant allele produced a ratio of the A and B reactions <0.2. A carrier of the mutation is detected by an absorbance ratio of the A and B reactions of >0.2 but <5 for the exon involved. For both the FinMajor and FinMinor mutation sites in exon 2, the absorbance ratio of the WT reaction to the mutant reaction (A/B) in samples from healthy individuals was significantly higher than the corresponding ratio in samples with the heterozygous genotype (P <0.001, Mann–Whitney U-test).

The 2.5th, 50th (median), and 97.5th percentile absorbance values of the WT genotype and the carriers of the FinMajor allele or the FinMinor allele along with the absorbance ratios of the WT reaction (A) to the mutant reaction (B) are shown in Table 2. Among 2004 Finnish blood samples, we found 35 carriers of NPHS1, of whom 34 individuals were carriers of the FinMajor allele and 1 was a carrier of the FinMinor allele. All of the samples showing the carrier genotype and 5% of the samples of the WT genotype were also analyzed using PCR-RFLP, and the results of these two separate assays were in perfect concordance. The calculated carrier frequency of the NPHS1 FinMajor allele in the Finnish population was 1:59 (95% confidence interval, 1.89–1.44) and that of the FinMinor allele was 1:2004 (95% confidence interval, 0 to 1:677). The rates of homozygosity for the NPHS1 FinMajor and FinMinor mutations predicted from the carrier frequencies observed in the present study were 1:13 900 (95% confidence interval, 1:31 700–1:7740) and 1:16 100 000 (95% confidence interval, 0 to 1:1 830 000), respectively, under the Hardy-Weinberg conditions. Because the FinMajor and FinMinor mutations account for 94% of all mutations causing NPHS1 in the Finnish population, the predicted combined carrier frequency of NPHS1 was 1:54 (95% confidence interval, 1:80–1:41) and the frequency of NPHS1 patients was 1:11 700 (95% confidence interval, 1:25 600–1:6720) in the population.

We also studied all of the carriers of the NPHS1 FinMajor mutation (n = 34), the single carrier of the NPHS1 FinMinor mutation, and two NPHS1 patients (one homozygous for the FinMajor mutation and the other homozygous for the FinMinor mutation) for the presence of the A3315 polymorphism. We found that 17 of 34 carriers of the FinMajor mutation were also carriers of the polymorphism and that 4 of 34 were homozygous for the polymorphism. Based on this result, the frequency of the polymorphism allele is lower among the carriers of the FinMajor mutation (37%, 95% confidence interval, 25–48%) than among the rest of the population. The carrier of the FinMinor mutation was also a carrier of the polymorphism, but neither of the NPHS1 patients carried the polymorphism.

**Discussion**

The prenatal diagnosis of NPHS1 has been based on the clinical features of the fetus and measurement the α-fetoprotein concentration in maternal serum or amniotic fluid. The two most common mutations that cause NPHS1 cover >94% of the NPHS1 chromosomes in the Finnish population (6), thus enabling the specific prenatal or neonatal diagnosis of 88% of NPHS1 cases by detection of those two mutations. Moreover, detection of the carriers of these mutations can be used to identify couples at risk of having a child affected by NPHS1. The FinMajor and FinMinor mutations seem to be rare in other populations because among 35 NPHS1 patients from North America, North Africa, and Europe, only 2 Swedish and 4 Finnish patients had the mutations (9).

PCR-RFLP can be used for the detection of mutations that cause the elimination or creation of a restriction site (10). The detection of the FinMinor allele is achieved by detecting the elimination of the naturally occurring AvaII site, but the detection of the FinMajor allele requires creation of an artificial restriction site for TaqI enzyme by the addition of a mismatch to the PCR primer. PCR-RFLP is a highly specific method, but the lack of automation makes it inefficient for analyzing large sets of samples.

PCR-OLA assay has many advantages in the detection of characterized mutations in large-scale screening programs (8). The use of several haptens enables the detection of several mutations in a single well, further increasing the throughput of the assay (11). Dual-color PCR-OLA with ELISA-based detection has been used previously for the genotyping of diallelic sequence variations (11) and the detection of mutations in the human immunodeficiency virus (12). This methodology has now been applied to the detection of the FinMajor allele located...
FinMinor mutations are needed to accurately determine the allele frequencies in the studied population, assuming that 94% of the NPHS1 carriers in the studied allele is the prominent NPHS1 polymorphism. Despite successful amplification in PCR, a weak or absent signal was detected in some samples for the WT allele at the FinMinor site when primers based on the WT sequence at that region (6) were used. The reason for the failure in OLA was found to be a neutral polymorphism (G3315A) in the region of reporter probe. The polymorphism affects the third base of a codon and has no effect on the amino acid sequence. However, it precludes the ligation of the oligonucleotides in OLA. This polymorphism has a very high prevalence in the studied population (allele frequency of 49%; 95% confidence interval, 45–54%). To obtain a positive signal for all of the amplified samples, equal amounts of two different reporter probes, one specific for the WT sequence (G3315) and the other specific for the sequence containing the A3315 polymorphism, were used in both ligation reactions.

The analysis of the A3315 polymorphism allele among the carriers of the NPHS1 FinMajor allele revealed that its frequency is lower among the carriers of the FinMajor mutation (37%; 95% confidence interval, 25%–48%) than among those negative for the mutation. DNA samples from NPHS1 patients homozygous for the FinMajor and the FinMinor mutations are needed to accurately determine the polymorphism frequency on mutant chromosomes.

The detected carrier frequency (1.59; 95% confidence interval, 1.89–1.44) for the NPHS1 FinMajor allele was similar to the previously reported frequency (6). This is higher than the carrier frequency of the major mutations that cause aspartylglycosaminuria or infantile neuronal ceroid lipofuscinosis in the Finnish population (1.94–1.83 and 1.95, respectively) (13–15). The detected carrier frequency for the FinMinor allele, 1.2004 (95% confidence interval, 0 to 1.677), is lower than the frequency reported in a study of 49 patients (6). This may be attributable to local differences in the occurrence of the NPHS1 alleles in the Finnish population. Our results show that the FinMajor allele is the prominent NPHS1 allele in eastern Finland, where the samples for this study were collected. The calculated frequency of NPHS1 carriers in the studied population, assuming that 94% of the NPHS1 mutations are FinMajor and FinMinor mutations (6), would be 1.54 (95% confidence interval, 1.80–1.41), predicting an incidence of 1:11 600 (95% confidence interval, 1:25 600–1:6720) for NPHS1. This incidence corresponds to earlier studies among Finnish population (1, 2), but for reasons as yet unknown, it is much lower than the incidence of NPHS1 cases (1:2400) detected in eastern Finland by prenatal α-fetoprotein screening (4, 5). These data give further evidence that there may be local differences in the distribution of the NPHS1 alleles and that other mutations may be present in the population of eastern Finland.

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References