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Structure of the Gene for Congenital Nephrotic Syndrome of the Finnish Type (NPHS1) and Characterization of Mutations

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Summary

Congenital nephrotic syndrome of the Finnish type (NPHS1) is an autosomal recessive disorder that is caused by mutations in the recently discovered nephrin gene, *NPHS1* (AF035835). The disease, which belongs to the Finnish disease heritage, exists predominantly in Finland, but many cases have been observed elsewhere in Europe and North America. The nephrin gene consists of 29 exons spanning 26 kb in the chromosomal region 19q13.1. In the present study, the genomic structure of the nephrin gene was analyzed, and 35 NPHS1 patients were screened for the presence of mutations in the gene. A total of 32 novel mutations, including deletions; insertions; nonsense, missense, and splicing mutations; and two common polymorphisms were found. Only two Swedish and four Finnish patients had the typical Finnish mutations: a 2-bp deletion in exon 2 (Fin_{major}) or a nonsense mutation in exon 26 (Fin_{minor}). In seven cases, no mutations were found in the coding region of the *NPHS1* gene or in the immediate 5′-flanking region. These patients may have mutations elsewhere in the promoter, in intron areas, or in a gene encoding another protein that interacts with nephrin.

Introduction

Congenital nephrotic syndrome of the Finnish type (CNF, NPHS1; MIM 256300) is an autosomal recessive disease characterized by massive proteinuria, detectable in utero, a large placenta (>25% of the newborn's weight), and marked edema (Hallman et al. 1956). The onset of nephrotic syndrome usually occurs within the first days of life and always before 3 mo of age. NPHS1 previously resulted in death before age 2 years, but it can now be treated by renal transplantation, without the development of extrarenal symptoms (Holmberg 1991, 1995).

We previously localized the *NPHS1* gene to chromosome 19q13.1 (Kestilä et al. 1994; Männikkö et al. 1995) and recently identified the actual gene (Kestilä et al. 1998). The *NPHS1* gene is 26 kb in size and contains 29 exons. The gene product, termed "nephrin," is a tentative membrane protein that is not notably expressed in extrarenal tissues. In the kidney, nephrin expression is observed only in visceral epithelial cells of the glomeruli, as determined by in situ hybridization, indicating its importance for the development or maintenance of the glomerular filtration barrier. Four different mutations have been reported in numerous Finnish and two North American families (Kestilä et al. 1998). Most (78%) Finnish *NPHS1* chromosomes and one North American patient had a 2-bp deletion in exon 2 (Fin_{major}) that caused a frameshift and a translation stop at the end of exon 2. A nonsense mutation in exon 26 (Fin_{minor}) was observed in 16% of Finnish *NPHS1* chromosomes. One American patient was a heterozygote for the Fin_{major} mutation, carrying a single base insertion in exon 24 of the other allele. Another North American patient was a homozygote for a 2-bp insertion in exon 10.

NPHS1 belongs to the Finnish disease heritage, which refers to enrichment of a disease in the isolated Finnish population (Nevanlinna 1972). Almost half of the known NPHS1 cases worldwide have been diagnosed in Finland (Norio et al. 1966). The incidence of NPHS1 is ~1:10,000 newborns in Finland (Huttunen 1976), but the incidence is considerably lower in other countries. For example, Albright et al. (1990) estimated an incidence of ~1:50,000 in North America. Cases of NPHS1

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Table 1

Deletion, Insertion, Nonsense, and Splicing Mutations in the *NPHS1* Gene in Congenital Nephrotic Syndrome Patients.

Exon	Nucleotide Change	Mutation Code	Effect on Coding Sequence	Effect on Protein[a]	Patients	Patient Origin	Comment
Frameshift mutations:							
2	nt121(delCT)	nt121(del2)	Frameshift	Trunc. 90-res. protein	6, 16, 18[b], 19, 20, 27[c]	Finland, Sweden	Fin_{major}
5	nt534(delG)	nt534(delG)	Frameshift	Trunc. 180-res. protein	13	North America	
10	nt1275(delC)	nt1275(delC)	Frameshift	Trunc. 435-res. protein	24[c]	North America	
19	nt2607(insCC)	nt2607(ins2)	Frameshift	Trunc. 851-res. protein	13	North America	
24	nt3250(insG)	nt3250(insG)	Frameshift	Trunc. 1,096-res. protein	11	North America	
Nonsense mutations:							
15	nt1954(C→T)	Q652X	Gln652→stop	Trunc. 652-res. protein	10	Sweden	
26	nt3325(C→T)	R1109X	Arg1109→stop	Trunc. 1,109-res. protein	16	Sweden	Fin_{minor}
27	nt3478(C→T)	R1160X	Arg1160→stop	Trunc. 1,160-res. protein	5	Italy	
Splicing mutations:							
Intron 4	nt526+5(G→C)	nt526+5(G→C)	Intron 4+5(G→C)	Splice-site mutation	10	Sweden	
18	nt2335-1(G→A)	nt2335-1(G→A)	Exon18-1(G→A)	Splice-site mutation	12, 22, 26[c]	North America, England	
Nonframeshift deletions and insertions:							
Promoter	nt-489(del$(GA)_4$)	nt-489del$(GA)_4$			8	North America	
Promoter	nt-489(delGA)	nt-489(delGA)			9	North Africa	
4	nt514(delACC)	ΔT172	Deletion of Thr172		17	Netherlands	
6	nt603(delCACCCCGG, insTT)	nt603(del8, ins2)	Tyr205, Pro206, Arg207→Ile205		4	Turkey	

[a] Trunc. = truncated, and res. = residue.
[b] Three heterozygous mutations found.
[c] Mutations found in only one allele.

Table 2

Missense Mutations in *NPHS1* Gene Found in Congenital Nephrotic Syndrome Patients

Exon	Nucleotide Change	Mutation Code	Effect on Protein	Patients	Patient Origin
2	nt191(G→C)	W64S	Trp64→Ser	19	Finland
4	nt512(T→A)	I171N	Ile171→Asn	2	Turkey
4	nt518(T→A)	I173N	Ile173→Asn	14	France
7	nt808(G→T)	G270C	Gly270→Cys	22	England
9	nt1048(T→C)	S350P	Ser350→Pro	14	France
9	nt1096(A→C)	S366R	Ser366→Arg	1, 11	Croatia, North America
9	nt1099(C→T)	R367C	Arg367→Cys	21[a]	France
9	nt1102 (C→T)	P368S	Pro368→Ser	9[b]	Netherlands
9	nt1126 (C→G)	L376V	Leu376→Val	9[b]	Netherlands
10	nt1223(G→A)	R408Q[c]	Arg408→Gln	18[a], 23 28[d]	Finland, North America
11	nt1384(G→A)	C465Y	Cys465→Tyr	18[a]	Finland
12	nt1583(G→T)	C528F	Cys528→Phe	15	France
14	nt1829(T→A)	L610Q	Leu610→Gln	15	France
14	nt1868(G→T)	C623F	Cys623→Phe	23	North America
16	nt2171(C→G)	S724C	Ser724→Cys	21[a]	France
17	nt2227(C→T)	R743C	Arg743→Cys	20	Finland
18	nt2404(C→T)	R802W	Arg802→Trp	17	Netherlands
18	nt2405 (G→C)	R802P	Arg802→Pro	12	North America
18	nt2417(C→A)	A806D	Ala806→Asp	3	Morocco
18	nt2491(C→T)	R831C	Arg831→Cys	25[d]	North America
27	nt3418(C→T)	R1140C	Arg1140→Cys	21[a]	France

[a] Three heterozygous mutations found.
[b] Patient is homozygous for P368S and heterozygous for L376V.
[c] Mutation found also in 4/30 controls as a heterozygous change.
[d] Mutation found only in one allele.

have also been reported in several other countries (Mahan et al. 1984; Mauch et al. 1994). Linkage to the NPHS1 locus has been shown by Männikkö et al. (1996) and Fuchshuber et al. (1996) in non-Finnish NPHS1 cases, indicating a more general involvement of the nephrin gene in hereditary nephrotic syndromes. In the present article, we describe in detail the structure of the human *NPHS1* gene, primers for amplification of its exon regions, and several novel mutations in the gene in CNF patients from Europe, North America, and North Africa.

Subjects, Material, and Methods

Structure of the NPHS1 *Gene*

Cosmid clone R33502 (AC002133) from chromosomal region 19q13.1 containing the *NPHS1* gene encoding nephrin was sequenced in its entirety. Potential coding areas were searched with several exon-prediction programs (e.g., GRAILII [Uberbacher and Mural 1991], FEXH, HEXON, FGENEH [Solovyev et al. 1994], and GENSCAN [Burge and Karlin 1997]), and primers were designed to create cDNAs from fetal kidney mRNA by reverse transcription PCR. A cDNA of 4.3 kb was sequenced and compared with the genomic sequence to obtain the gene structure. The SIGNAL SCAN program (Prestridge 1991) was used to analyze the promoter area.

Patients and Control Material

Genomic DNA was extracted according to standard laboratory protocol (Vandenplas et al. 1984) from peripheral blood leukocytes from 30 non-Finnish controls, 10 Finnish controls, and 35 NPHS1 patients and their parents from North America, Europe, and North Africa. The diagnosis of NPHS1 was made on the basis of a large placenta and severe proteinuria at birth, with nephrotic syndrome appearing during the first weeks of life, and, whenever possible, a renal histology compatible with NPHS1 was used for the diagnosis. Diffuse mesangial hypercellularity with an increase in mesangial matrix and focal dilatation of proximal tubules was observed in all patients. At least four of the patients (patients 3–5 and 8) were from consanguineous families (tables 1 and 2).

Southern Hybridization

DNA from 10 unrelated non-Finnish patients was digested with *Eco*RI restriction enzyme and electrophoresed in an agarose gel. DNA was blotted to a nitrocellulose filter, and the filter was hybridized with a

radioactively labeled 1.3-kb nephrin cDNA probe (corresponding to exons 1–10).

Mutation Screening

Exons of the *NPHS1* gene were amplified by PCR (Perkin-Elmer Cetus 9600) with primers designed for exons 1–29 (table 3), and the PCR products were sequenced in ABI 377 and ABI 310 sequencers (Perkin-Elmer). PCR reactions were performed in a total volume of 25 μl, which contained 20 ng of template DNA, 1 × AmpliTaq reaction buffer (Perkin-Elmer), 0.2 mM each nucleotide, 100 ng of primers, and 0.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer). The reactions were performed for 30 cycles, with denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. In the first cycle, denaturation was performed for 12 min, and in the last cycle, the extension lasted for 8 min. Both strands of the PCR products were then sequenced with dRhodamine and BigDye terminator sequencer chemistries (Perkin-Elmer).

Results

Structure of the NPHS1 *Gene*

The structure of the *NPHS1* gene was predicted from the genomic sequence (cosmid clone R33502, Genbank accession number AC002133) by several exon-prediction programs. On the basis of these results, the coding regions of the *NPHS1* gene were amplified from fetal kidney cDNA, and the exon-intron structure was determined by comparing the sequence of the 4.3-kb human nephrin cDNA with the genomic sequence (Kestilä et al. 1998). The gene was shown to span ~26 kb, proceeding 5′ to 3′ from the telomere toward the centromere of chromosomal region 19q13.1. The gene contains 29 exons, with sizes ranging from 25 to 216 bp (fig. 1 and table 4). With the exception of exon 23, which has an unusual donor site starting with GC, the exons have the conventional acceptor (AG) and donor sites (GT). The exon-prediction program GENSCAN could correctly predict all exons except exon 23. The codon for the translation initiator methionine is in the first exon, and the translation stops at TAA in exon 29.

Initial studies on the transcription start site revealed a primer extension product starting 157 bp upstream of the starting methionine (data not shown). The sequence of part of the *NPHS1* promoter area and 5′ UTR is shown in figure 2. In this area, there is no TATA box, but there are two putative GATA factor binding sites, three NF-1 sites, and one AP2 consensus sequence.

Nephrin, the *NPHS1* gene product, is a putative transmembrane receptor that contains eight immunoglobulin C2-like motifs (Kestilä et al. 1998). The first exon codes for the signal peptide, and exons 2–20 encode the region containing the immunoglobulin motifs. Each immunoglobulin motif is encoded by two exons, except motif Ig2, which is encoded by three exons. Exons 22 and 23 code for a fibronectin type III–like domain, and exon 24 codes for the transmembrane domain. Exons 25–29 encode the putative cytosolic domain and the 3′ UTR.

Analysis of the NPHS1 *Gene Region in NPHS1 Patients*

Southern analysis of DNA from NPHS1 patients did not reveal large genomic rearrangements. Consequently, we searched for mutations by PCR amplification and

Table 3

PCR Primer Sets for Amplifying Exons of *NPHS1*

Exon	5′ Primer	3′ Primer	Product Size (bp)
5′ UTR	gctgactctgccagtgcctg	cagggccatcacaggtcccc	643
1–2	gagaaagccagacagacgcag	agcttccgctggtggct	491
3–4	agccaccagcggaagct	ctcccttcccactccagagg	530
5	cagaatctatcttgcggggag	catggggaaaattaggggtcaag	187
6–7	tctccctgactccccaaatttc	ctcaggactggctcccagac	544
8–9	gacagtggggtctgggagccagtcctgag	gagtcatgccctcagcccc	623
10–11	cacgatggataggggtgctg	cctggtccttcccccacatt	465
12–13	aacccagtgggcagggtagggg	gacatgcgtggaggggcga	668
14	cctagtgcctctccagcc	gagtagtttagggtcaagaagg	288
15–16	cctgatctccaatctgtccttg	ccacaatgggcaaggttccttg	484
17	cacccagacctgtctgggcc	gtccccactcccaaggaactc	257
18–19	gaggctacagaagggacaatttg	gctggaggtccagacctggg	561
20	ggatggatgcatagatgattcc	caatcagggatgtgggaatg	297
21–22	cctggacagaatcttctggaatt	cctacacatcctctgaggaatac	487
23	gaggctgagaaatatttaaagcttat	gagaccaggaggttccattct	188
24–26	ctcggggagacccacccc	cctgatgctaacggcagggc	611
27–28	tgcccttccgggcacagtgg	tacaagcaataggaggtaggc	438
29	cagatcttcaatgaagacctaca	gagacagaatctcgctctg	747

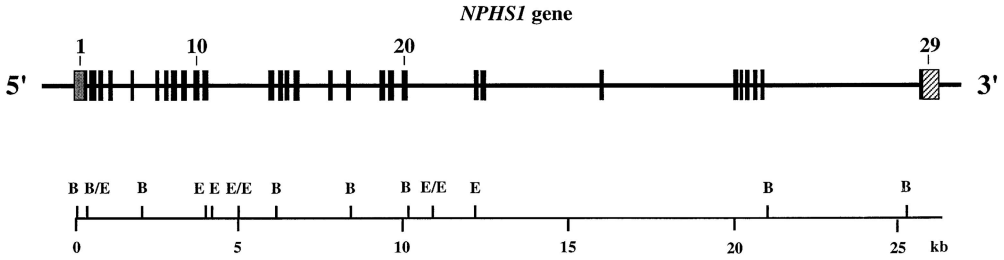

Figure 1 Schematic structure of the human *NPHS1* gene. *Top,* Exons are indicated by black rectangles; introns and flanking sequences are indicated by rectangles with diagonal lines. The exons are numbered. *Bottom, Eco*RI (E) and *Bam*HI (B) map of the gene and scale in kilobases.

direct sequencing of all 29 exon regions. DNA from 35 European, North American, and North African patients with suspected NPHS1 was analyzed. The mutations are summarized in tables 1 and 2. In 23 patients, changes were found in both alleles, and in 5 patients, changes were found in only one allele. No mutations were found in 7 patients, despite analysis of the promoter region and all 29 exon regions. DNA samples from parents were also studied to analyze the mode of inheritance: a pattern of Mendelian inheritance was observed in all cases (data not shown).

Patients 1–8 (tables 1 and 2) were homozygous for their specific mutations. Three of the homozygous mutations result in single amino acid substitutions (patients 1–3), one results in deletion of three residues and insertion of a new residue (patient 4), and one results in a truncated polypeptide chain due to introduction of a premature stop codon (patient 5). One Swedish patient (patient 6) was homozygous for the Fin_{major} mutation. Two patients (patients 7 and 8) had a deletion (8 and 2 bp, respectively) in a GA repeat sequence of the promoter region. Patient 9 was homozygous for one mutation (P368S) and had another mutation (L376V) in one allele. Patients 10–23 were heterozygotes for a variety of mutations. Of interest, two patients (patients 18 and 21) had three nucleotide changes. Five patients (patients 24–28) had a mutation in only one allele. The most common missense change was Glu117→Lys, which was found either homozygously or heterozygously in nine patients (table 5); however, this variant was also found heterozygously in 8 of 20 individuals not having NPHS1 and homozygously in 3 controls. This confirms that this is not a disease-causing mutation.

Deletions and Insertions

There were six patients (patients 6, 16, 18–20, and 27) with the Fin_{major} mutation (2-bp deletion), one (patient 13) with a two-base insertion, two (patients 13 and 24) with different single base deletions, and one (patient 11) with a single base insertion; all of these mutations lead to frameshifts and truncated proteins (table 1). The sizes of truncated proteins are shown in figure 3. Also, one 3-bp deletion results in the loss of Thr172 (patient 17). One mutation with a deletion of eight bases and insertion of two bases in exon 6 was also found (patient 4). This mutation results in the deletion of Thr205, Pro206, and Arg207 and an insertion of isoleucine in the same site within the Ig2 motif.

Splice-Site and Nonsense Mutations

Three patients (patients 12, 22, and 26) had an identical splice-site change in exon 18, and one (patient 10) had a G→C substitution in the splice-site consensus sequence of intron 4, changing a conserved G at position +5, which is necessary for correct splicing (Senapathy et al. 1990) (table 1). Three different single base nonsense mutations leading to introduction of a translation stop codon were also found (patients 5, 10, and 16).

Missense Mutations

The 23 missense mutations (62%) causing substitution of a single amino acid are summarized in figure 3 and table 2. These amino acid changes were scattered throughout the nephrin polypeptide chain: 21 were in the immunoglobulin motifs of the extracellular domain, one was between immunoglobulin motifs, one was in the transmembrane domain, and one was in the cytosolic domain (fig. 3). Nine of the mutations involved either the loss or introduction of a cysteine residue (i.e., mutations that can either alter the secondary structure of the protein or result in new intra- or interchain disulfide bonds that affect the function of the protein). The other mutations result mainly in changes in the charge or size of the amino acid. Of interest, one patient (patient 21) had three different amino acid changes in heterozygous forms (table 2). Each of them was an amino acid substitution to a cysteine residue. In this patient, two of the changes were in the immunoglobulin domains (maternal

Table 4

Exon-Intron Boundaries and Sizes of Exons and Introns in the Human *NPHS1* Gene

Exon	Exon/Intron Boundaries				Exon Size	Intron Size
1	5′ UTR	ATG GCC CTG...	...ACT GAA G	gtgagtggga	5′ UTR + 78	87
		M A L	T E G			
2	gtgtcctcag	GC CTG GCG...	...GCT AGA G	gtaagggatc	216	71
		L Q	A R G			
3	ccccctgcag	GT GAA TTC...	...ATC CTG G	gtatgggtgag	124	172
		E F	I L V			
4	ctctcaccag	TT CCT CCC...	...CTC CTG A	gtgagtgtgg	129	515
		P P	L L S			
5	ctgccccaag	GT GGA CAG...	...ACA GCC AG	gtgtggaaac	82	710
		G Q	T A R			
6	ctcatcccag	G GTG ACA...	...GTT CTG T	gtgaggcagt	104	186
		V T	G K			
7	tatcccccag	TC CCT CCA...	...TGG CTG AAG	gtgagggcaa	128	87
		P P	W L K			
8	ctgtctccag	AAT GGC CAG...	...GTC ACC T	gtgagtcctg	173	181
		N G Q	V T F			
9	gtccctgcag	TT CCC CCT...	...GTC ATG GAT	gtgaggcggg	158	239
		P P	V M D			
10	tccctgccag	GGA CTG CAT...	...GTA AAA T	gtgagcccct	145	87
		G L H	V K Y			
11	tccccgccag	AT CCC GCC...	...TGG TAC AAG	gttggtgcca	125	1,846
		P A	W Y K			
12	cggcccgtag	GAC TCG CGC...	...GTG CAG T	gtgagggctc	187	209
		D S R	V Q F			
13	cgtctcccag	TT CCC CCA...	...GGG GAG AG	gtgggagtgc	136	128
		P P	G E R			
14	ggttccacag	G CTG GAG...	...GTA CTG T	gtatgtgccc	173	908
		L E	V L Y			
15	ccctctgaag	AC CGT CCA...	...AGT CCA G	gtgagggaag	141	75
		R P	S P A			
16	ccttccccag	CG GGC GGC...	...GTG CAC T	gtgagccccg	141	509
		G G	V H Y			
17	cttccaccag	AT GCT CCC...	...GAG AGA CTG	gtgaggatcc	122	921
		A P	E R L			
18	gggcctccag	GGA GAA GAT...	...GTC AGA T	gtgggtatta	172	98
		G E D	V R F			
19	cccctcccag	TT GCC CCC...	...GAT CCC AG	gtgagcccaa	157	257
		A P	D P R			
20	ttctctctag	G TAC ACG...	...AGC ATC A	gtatggaggg	152	2,108
		Y T	S I S			
21	ctccccatag	GC CGC CCT...	...TGC ATC AG	gtgggtcctt	112	77
		R P	C I R			
22	tgtcctgcag	G TAT GAG...	...ACC CCA G	gtgggaaggga	182	3,476
		Y E	T P G			
23	caccccccag	GT CTC CAC...	...CCT TCA G	gcaagtcctca	57	3,942
		L H	P S G			
24	ccacccctag	GA CCC TCG...	...GCT GAG G	gtgaggagaga	120	142
		P S	A E G			
25	tttccttcag	GC ATC TCA...	...GAG GCA GG	gtaagtgggga	25	104
		I S	E A G			
26	atcctggaag	G TCG GAA...	...AGC TCC ACG	gtaagtgggggg	76	149
		S E	S S T			
27	tggggaacag	GTC AGC ACA...	...TCC CGA G	gtgagttagga	94	96
		V S T	S R G			
28	ccaactccag	GT TTC ACA...	...GTG CAG ATG	gtgagaagctt	113	4,198
		F T	V Q M			
29	tgcctgtcag	GGA CCC TGG...	...CAT CTG GTG	taa 3′ UTR	129 + 3′ UTR	
		G P W	H L V	*		

NOTE.—Intron sequences are shown in lowercase letters. Exon sequences are shown in uppercase letters, with the amino acid residues shown below (one-letter codes). The translation stop codon taa is indicated by an asterisk (*). Exon and intron sizes given are in base pairs.

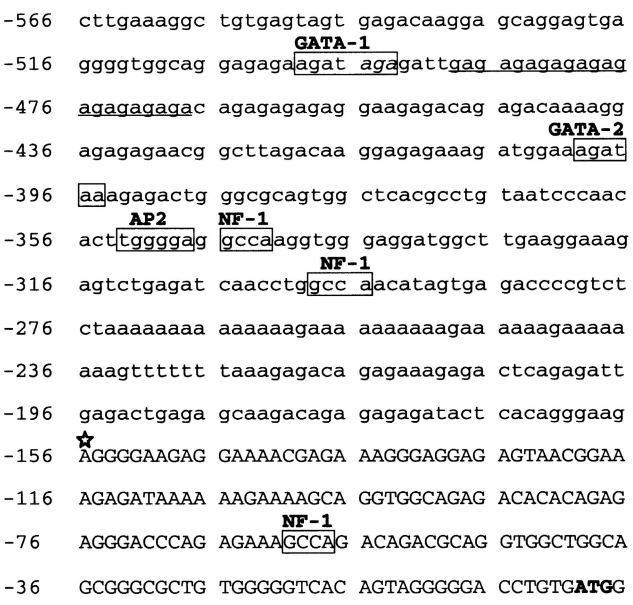

Figure 2 Sequence of the immediate upstream region and 5′ end of the *NPHS1* gene. The putative 5′ end of the transcript is shown with uppercase letters. A star indicates the transcription initiation site as detected by primer extension. The consensus sequences for transcription factors GATA-1, GATA-2, NF-1, and AP-2 are boxed. The ATG codon for the initiator methionine is indicated by boldface type, and the GA-rich region containing a mutation in two patients (see text) is underlined. The nucleotides are numbered so that the first nucleotide of the codon for the initiator methionine is +1.

and paternal inheritance, respectively), and one was in the cytosolic domain (maternal inheritance) of nephrin.

Sequence Variants in Control Individuals

All together, 23 sequence variants resulting in amino acid substitutions in the nephrin polypeptide chain were found in NPHS1 patients. To examine whether these variants are normal polymorphisms or disease-causing mutations, we sequenced corresponding codons from the DNA of 30 unrelated non-Finnish individuals. These analyses revealed three sequence variants, of which two were found as a homozygous change in controls (table 5). The most frequent sequence variant (in 22/60 analyzed chromosomes) leads to a Glu117→Lys change in the Ig1 motif. This was found as a homozygous change in five control subjects and is therefore most likely a common polymorphism. Also, a sequence change leading to the amino acid change Asn1077→Ser in exon 24 was observed in both alleles of one Finnish control. This mutation was also present as a heterozygous change in five NPHS1 patients (patients 17, 18, 19, 27, and 28). This change was observed to be inherited together with the Fin_{major} mutation. Four control subjects were heterozygotes for a mutation in exon 10 leading to an Arg408→Gln change found in three compound heterozygous patients (patients 18, 23, and 28) (table 2). Patient 18 also had two other mutations, Fin_{major} and Cys465Tyr.

Discussion

The present study provides the complete structure of the human nephrin gene (*NPHS1*) as well as information on sequences and PCR conditions facilitating mutation analysis of all 29 exons of the gene in individuals with NPHS1. Furthermore, we have identified a large number of novel biallelic mutations in the gene in NPHS1 patients, demonstrating the involvement of this gene in the disease. Surprisingly, several sequence variants were also detected in control individuals, indicating that this gene is quite susceptible to mutagenesis.

Except for exon 23, the 29 exons of the gene were shown to have conventional acceptor (AG) and donor (GT) sites; exon 23, which encodes part of the type III fibronectin domain, has a donor site starting with GC instead of GT. Sequencing of DNA from several individuals showed that this sequence is a normal feature of the gene. Although extremely rare, this donor site sequence has been described for a single exon in a few genes from different species (Shapiro and Senapathy 1987; Soininen et al. 1989; Senapathy et al. 1990). The potential significance of this donor site sequence is still unknown. Each of the immunoglobulin motifs of nephrin was shown here to be encoded by two exons, except for Ig2, which is encoded by three exons. Thus, the nephrin gene resembles the genes for a group of transmembrane cell adhesion proteins belonging to the immunoglobulin superfamily, such as the neural cell adhesion molecules N-CAM (Owens et al. 1987) and MUC18 (Sers et al. 1993), in which each immunoglobulin motif is encoded by two exons. In several other genes coding for proteins of the immunoglobulin superfamily, each immunoglobulin motif is encoded by a single exon (see Lawlor et al. 1990; Schrewe et al. 1990; Cybulsky et al. 1991; Nakano et al. 1991).

In this study, we identified 32 novel mutations in the *NPHS1* gene of Finnish and non-Finnish patients, making the number of known mutations in this gene 36. In addition, two common polymorphisms (Glu117→Lys and Asn1077→Ser) were found. We have done mutation analysis for 79 individuals with suspected NPHS1 and found mutations in both alleles of *NPHS1* in 67 patients and in only one allele in 5 patients, by sequencing all exon regions and the immediate promoter, where two sequence changes were detected. This corresponds to a mutation detection rate of ~88% by the PCR amplification and direct DNA sequencing approach. It is possible that the few mutations yet to be identified in patients in our study are located in gene regions containing important regulatory elements of the *NPHS1* gene.

Table 5

Sequence Variants in the *NPHS1* Gene in Control Individuals

Nucleotide Change	Exon	Effect on Coding Sequence	Heterozygote or Homozygote	No. of Control Subjects (n = 30)
nt349(G→A)[a]	3	Glu117→Lys	Heterozygote	12
			Homozygote	5
nt3230(A→G[b]	24	Asn1077→Ser	Homozygote	1
nt1223 (G→A)[c]	10	Arg408→Gln	Heterozygote	4

[a] Present also as a homozygous change in three NPHS1 patients (patient 9; table 2) and as heterozygous change in six NPHS1 patients (patients 11, 13, 15, 16, 24, and 28; tables 1 and 2). Also found in fetal kidney cDNA library.

[b] Present also as a heterozygous change in five NPHS1 patients (patients 17, 18, 19, 27, and 28; tables 1 and 2).

[c] Present also as a heterozygous change in three NPHS1 patients (patients 18, 23, and 28; tables 1 and 2).

Although NPHS1 has been reported mainly in the homogeneous Finnish population (Norio et al. 1966), it is also present elsewhere (Mauch et al. 1994), and the present study suggests that mutations in the nephrin gene are a general cause of hereditary CNF. We have previously reported the presence of two main mutations, Fin_{major} and Fin_{minor}, in 94% of 98 known Finnish *NPHS1* chromosomes (Kestilä et al. 1998). The present study reveals that these two mutations are rare in other populations. These mutations were found to be homozygous or compound heterozygous in 44 of 49 Finnish patients studied, 4 being heterozygotes, with the second mutation unknown. In the present study, we identified four novel mutations in Finnish patients; thus, we have now found mutations in all but two of the analyzed Finnish *NPHS1* chromosomes. A total of 34 novel sequence variants were identified in 36 non-Finnish and Finnish patients with suspected NPHS1.

Mutations found in the promoter region of one North American and one North African patient (patients 7 and 8) are interesting. Patient 7, with a $(GA)_4$ deletion, did not have a typical course of NPHS1. She presented with proteinuria and mild edema at 4 mo of age. A kidney biopsy revealed mesangial proliferation without sclerosis. The nephrotic syndrome did not respond to steroid therapy. The child's edema was controlled with oral diuretics; albumin infusions were never required. Her renal function deteriorated gradually until age 5 years, when she received a kidney transplant. There has been no recurrence of nephrotic syndrome during 12 years of follow-up. This case indicated that *NPHS1* might be involved in a broader spectrum of disease than the classic Finnish type of NPHS1. However, in patient 8, who had the classical severe NPHS1 phenotype, there was only a deletion of GA in the same GA repeat sequence. Whether these sequence changes in the promoter actually cause disease remains to be shown.

The results of this study are of significant importance for the diagnosis of CNF. In the absence of DNA-based diagnostic methods, measurements of α-fetoprotein levels in the amniotic fluid or maternal blood have been used for prenatal diagnosis and abortions of NPHS1 fetuses in families at risk (Seppälä et al. 1976). In some cases, diagnosis of fetal NPHS1 has been made on the basis of increased α-fetoprotein levels during pregnancy, without a known family history for the disease. However, since elevation of α-fetoprotein levels is not specific for the CNF, DNA-based diagnosis should now be the preferred approach (Männikkö et al. 1997). Although the diagnostic procedure described in this study does not reveal 100% of the mutations causing CNF, most of the mutations can now be identified, especially in Finnish families (Kestilä et al. 1998; present study).

The large number of mutations described here can shed light on the nature of nephrin, the product of *NPHS1*. Small deletions and insertions, splice-site mutations, and some of the nonsense mutations in the gene result in frameshifts, premature stop codons, or abnormal mRNA splicing, resulting in nonfunctional protein. Since most of the truncated nephrin molecules lack the intracellular and transmembrane domains, they reveal only limited information on active domains of nephrin. In contrast, comparing missense mutations that lead to disease with those resulting in neutral amino acid substitutions can reveal significant information about functionally important domains and amino acid residues. Of the 36 mutations now known, 21 are missense mutations that result in amino acid substitutions. As shown in figure 3, most of the mutations causing amino acid substitutions are located in the extracellular domain of nephrin, one being in the transmembrane domain and one in the cytosolic domain. With the exception of mutation Ser724→Cys, which probably leads to the introduction of abnormal intrachain or interchain disulfide bonds, the mutations in the extracellular domain are within the IgC2-like motifs, indicating that these motifs are func-

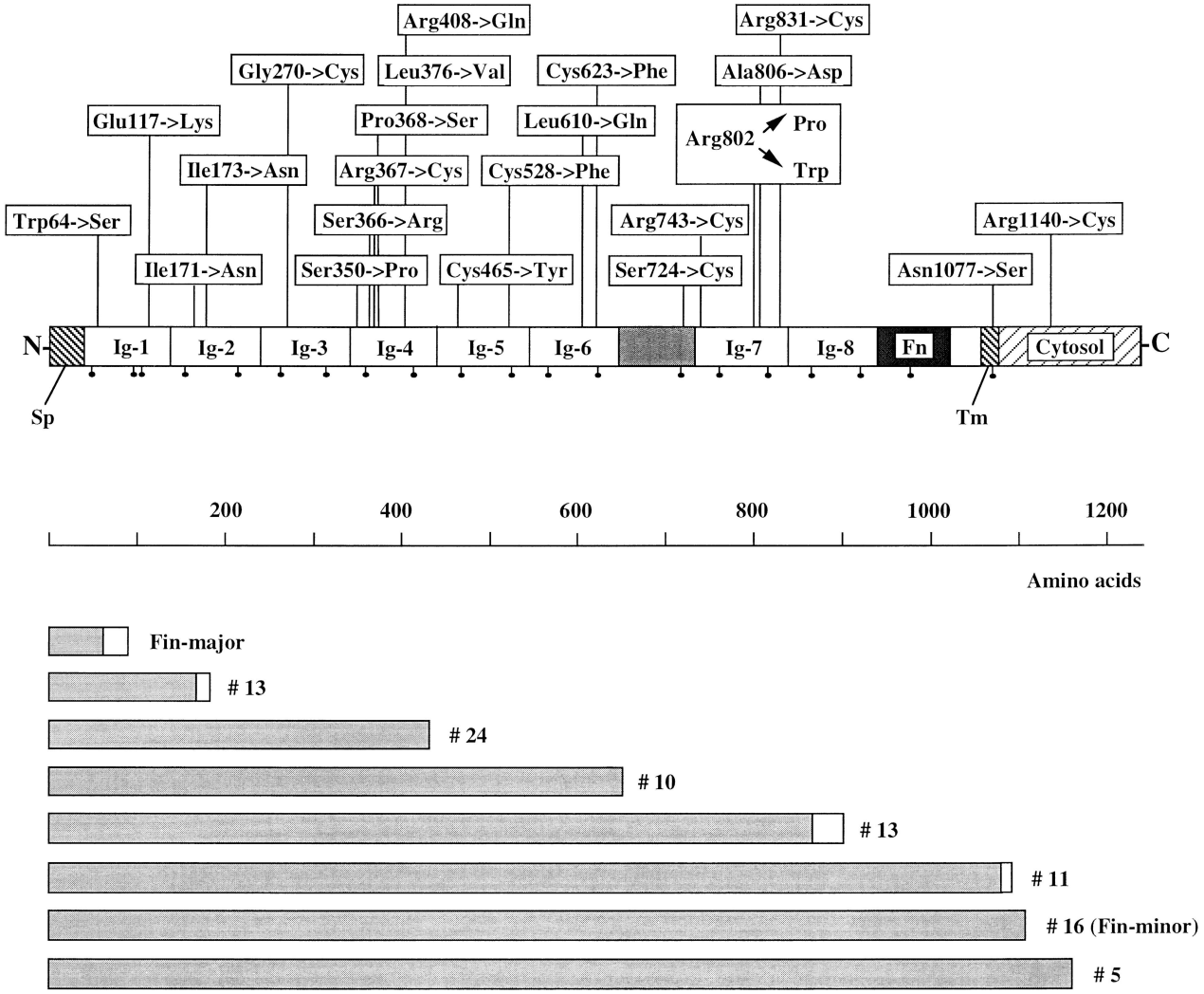

Figure 3 *Top,* Location of missense mutations and polymorphisms in the nephrin polypeptide chain. Locations of cysteines are indicated with blackened dots. *Middle,* Scale in amino acid residues. *Bottom,* Sizes of truncated nephrin proteins of NPHS1 patients. Sequence out of frame is shown with unblackened bars. The patient numbers (table 1) are shown on the right. Sp = signal peptide; Ig = immunoglobulin motif; Fn = fibronectin type III motif; and Tm = transmembrane domain.

tionally important and that their function is critically dependent on certain amino acid residues. Some clustering of mutations was observed. For example, about half of the amino acid changes occurred in motifs Ig4 (six mutations) and Ig7 (five mutations). One mutation was found in Ig3, and two each were found in Ig1, Ig2, Ig5, and Ig6, but no amino acid substitutions were observed in Ig8.

Future studies may reveal whether the clustering of mutations observed here reflects functional importance of the amino acid residues and motifs involved or is just the result of small sample size. Some of the amino acid substitutions were found heterozygously in a few of the control subjects. It is not clear whether these changes affect the function of the nephrin protein, but since the same amino acid changes were also present in patients with no other mutations found, we cannot exclude that these mutations actually cause disease. However, whether an amino change is a rare polymorphism or whether it causes disease will not be resolved until a functional assay is devised.

In conclusion, the results of this study clearly demonstrate the involvement of the nephrin gene in NPHS1. Whatever its function, the nephrin protein must be crucial for the selective filtration function of the kidney, because the consequence of nephrin absence or malfunction is massive proteinuria. The study also indicates that mutations in nephrin may be involved in proteinuric patients who do not exhibit the classic severe Finnish type of CNF. Consequently, there is a reason to examine

the involvement of nephrin in both genetic and acquired kidney disorders in which proteinuria is displayed.

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Electronic Database Information

Accession numbers and URLs for data in this article are as follows:

Genbank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for *NPHS1* cDNA, accession number AF035835, and for cosmid sequence AC002133, accession number R33502)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for NPHS1 [MIM 256300])

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