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Congenital nephrotic syndrome (NPHS1): Features resulting from different mutations in Finnish patients

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Congenital nephrotic syndrome (NPHS1): Features resulting from different mutations in Finnish patients.

Background. Congenital nephrotic syndrome (NPHS1) is a rare disease inherited as an autosomally recessive trait. The *NPHS1* gene mutated in NPHS1 children has recently been identified. The gene codes for nephrin, a cell-surface protein of podocytes. Two mutations, named Fin-major and Fin-minor, have been found in over 90% of the Finnish patients. In this study, we correlated the *NPHS1* gene mutations to the clinical features and renal findings in 46 Finnish NPHS1 children.

Methods. Clinical data were collected from patient files, and kidney histology and electron microscopy samples were re-evaluated. The expression of nephrin was studied using immunohistochemistry, Western blotting, and in situ hybridization.

Results. Nephrotic syndrome was detected in most patients within days after birth regardless of the genotype detected. No difference could be found in neonatal, renal, cardiac, or neurological features in patients with different mutations. Nephrin was not expressed in kidneys with Fin-major or Fin-minor mutations, while another slit diaphragm-associated protein, ZO-1, stained normally. In electron microscopy, podocyte fusion and podocyte filtration slits of various sizes were detected. The slit diaphragms, however, were missing. In contrast to this, a nephrotic infant with Fin-major/R743C genotype expressed nephrin in kidney had normal slit diaphragms and responded to therapy with an angiotensin-converting enzyme inhibitor and indomethacin.

Conclusions. The most common *NPHS1* gene mutations, Fin-major and Fin-minor, both lead to an absence of nephrin and podocyte slit diaphragms, as well as a clinically severe form of NPHS1, the Finnish type of congenital nephrotic syndrome.

Congenital nephrotic syndromes (CNS) form a heterogeneous group of diseases characterized by proteinuria shortly after birth [1]. NPHS1 (MIM 256300) is a distinct

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clinical entity among nephrotic syndromes [1]. It is an autosomal recessive disorder with an incidence of 1:8200 births in Finland [2, 3]; in other countries the incidence is clearly lower [4], so that approximately one half of the reported NPHS1 patients have been diagnosed in Finland.

The classic findings in NPHS1 include prematurity, large placenta, and proteinuria, which already begins in utero [5]. Nephrotic syndrome with heavy proteinuria, hypoalbuminemia, edema, and secondary manifestations is usually detected shortly after birth. Currently, children with NPHS1 are successfully treated with active protein and nutritional support, followed by bilateral nephrectomy and dialysis, and finally, by renal transplantation [6].

The gene for NPHS1 (*NPHS1* gene) has been localized to chromosome 19q13.1 [7, 8] and was recently identified as a novel 29-exon gene [9]. The protein product of this gene was termed nephrin. It is a transmembrane protein of the immunoglobulin superfamily containing 1241 amino acids. Nephrin is produced by glomerular podocytes, and recently, we localized nephrin at the slit diaphragm of the glomerular podocytes, suggesting that this structure and nephrin play an essential role in the normal glomerular filtration process [10].

The two most common mutations in the *NPHS1* gene have been named Fin-major and Fin-minor and are highly enriched in the Finnish population [9]. Sixty-five percent of the Finnish patients were homozygous for the Fin-major mutation, and 8% were homozygous for Fin-minor. Sixteen percent of the patients were compound heterozygous. Eight percent of the patients were Fin-major heterozygotes with missense mutations in the other chromosome, and one patient had three missense mutations. The Fin-major mutation results in a frameshift and a stop codon in exon 2 and a truncated protein of only 90 amino acids. Fin-minor mutation is a nonsense mutation in exon 26 and leads to a truncated 1109-residue protein.

In non-Finnish patients, a great variety of mutations have been described recently, including insertions, deletions, and nonsense and missense mutations [11]. These mutations were scattered along the entire *NPHS1* gene.

The clinical features of *NPHS1* children were thoroughly studied in the early 1970s [5]. Since then, the diagnostic methods and the management of these children have changed dramatically. This and the discovery of the genetic basis of this disease prompted us to perform a systematic analysis of the clinical manifestations and renal pathology of the Finnish *NPHS1* patients. This seemed especially interesting since the two most common mutations, Fin-major and Fin-minor, affect the opposite ends of the nephrin molecule.

METHODS

Genetic analysis

The identification of the *NPHS1* gene and an analysis of the mutations of *NPHS1* children have been described [9]. The total of 46 patients studied included 29 patients homozygous for the Fin-major mutation, 4 patients homozygous for the Fin-minor mutation, and 8 compound heterozygotes for these mutations. Three patients had a Fin-major mutation in one and an identified missense mutation in the other gene. One patient had a Fin-major mutation in one gene and probably a splice mutation in the other gene. Finally, one patient had three missense mutations (Fig. 1).

Patient management

The treatment of children with *NPHS1* occurred in three stages, as has been described earlier [1]. Briefly, after nephrosis was diagnosed, the patients were supplemented with daily albumin infusions and a high-calorie diet. The medication included thyroxin, sodium warfarin, and prompt antibiotic therapy for septic infections. Bilateral nephrectomy was performed when the children weighed about 7 kg and peritoneal dialysis was started. Renal transplantation either from a parent or from a cadaver donor was done when the patient weighed at least 9 kg [12].

Collection of clinical data

The clinical symptoms and signs as well as laboratory values during the nephrotic stage were analyzed retrospectively. These included birth weight and height, placental weight, gestational age, the time of recognition of the disease, blood, and urine biochemistry. The amount of protein loss was evaluated by recording urine protein measurements and the albumin substitution required at the age of three to six months. Neurological status, findings on electroencephalography (EEG), and computed tomography (CT) of the brain were recorded. The results of cardiac examinations were analyzed.

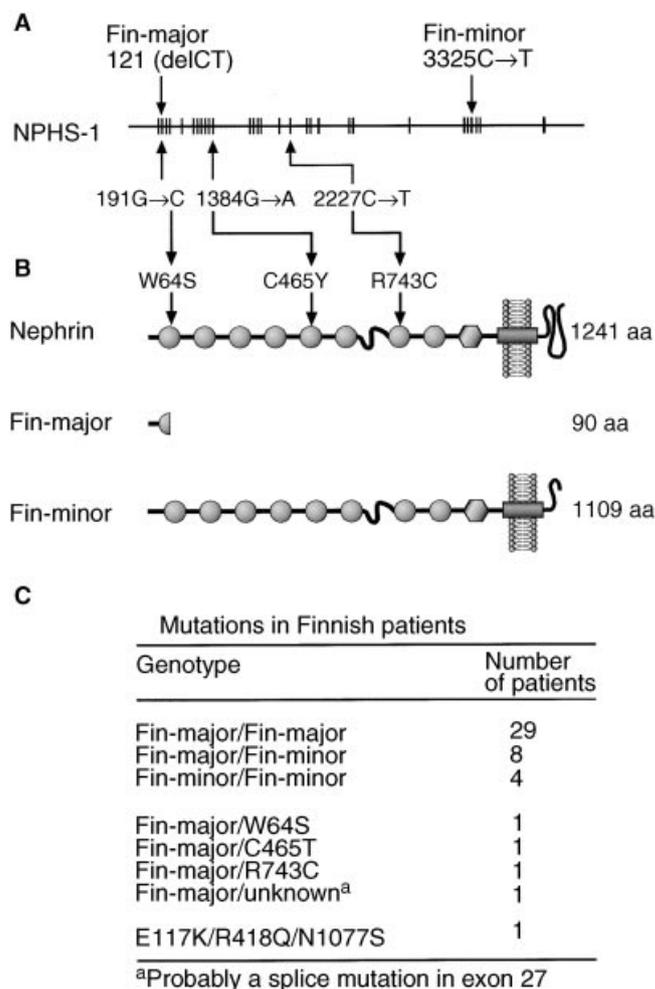


Fig. 1. *NPHS1* gene and mutations in Finnish patients. (A) *NPHS1* gene. Fin-major mutation is a 2 bp deletion in exon 2, and Fin-minor mutation is a nonsense mutation in exon 26. The three missense mutations are also indicated. (B) Nephtrin molecule. Fin-major mutation leads to a truncated protein of 90 amino acids and Fin-minor to a truncated protein of 1109 amino acids. W64S is a change of tryptophan to serine in Ig-1. C465T is a change of cysteine to tyrosine in Ig-5, and R743C is a change of arginine to cysteine in Ig-7. (C) Genotypes of the Finnish *NPHS1* patients included in this study.

Light and electron microscopy of kidney samples

Histologic slides of the nephrectomized kidneys between the years 1986 and 1998 were collected and re-examined. Forty-four cases were obtained. The ages of the patients at nephrectomy ranged from 4 months to 3.7 years, most of the nephrectomies (36 out of 44) being performed between 6 and 18 months. Abnormalities of glomeruli, tubuli, and blood vessels and interstitial fibrosis and inflammation were recorded. The number of obsolescent glomeruli, extension of tubular microcysts, interstitial fibrosis, and inflammation were arbitrarily graded from nil to 3+. The histology score was obtained by adding these together (Table 2).

Electron microscopy was performed according to stan-

Table 1. Neonatal and nonrenal characteristics in NPHS1 children with different mutations^a

Finding	Mutation			
	Major/major N = 29	Major/minor N = 8	Minor/minor N = 4	Others ^b N = 5
Neonatal				
Gestational age <i>weeks</i>	36.3 (32.7–39.4)	36.4 (35.0–37.7)	35.1 (32.0–37.0)	37.9 (36.0–39.4)
Birth weight <i>g</i>	2615 (1620–3410)	2540 (2010–3040)	2010 (1450–2270)	2657 (1840–3480)
Birth height <i>cm</i>	47 (43–52)	48 (46–49)	45 (43–46)	48 (45–50)
ISP %	38.6 (21.3–56)	38.0 (30–46)	47.5 (39.6–59)	40.5 (37–58)
Neurological				
Hypotonia	26/28 ^c	5/7 ^c	4/4 ^c	4/5 ^c
EEG abnormalities	10/26	2/7	1/4	0/4
CT/MRI abnormalities	12/26	4/7	1/4	1/4
Cardiac findings ^d	6/26	2/7	1/4	3/5

^a Results are given as means and range

^b These included three patients with Fin-major in one gene and missense mutation in the other gene, one patient with three missense mutations, and one patient with Fin-major mutation in one gene and a probable splice mutation in the other gene (Fig. 1)

^c Number of patients with the finding/total number of patients with adequate data

^d Cardiac findings include mild pulmonary stenosis (5 patients), cardiac hypertrophy (3 patients) and atrial septal defect (1 patient)

dard procedures. Samples from nephrectomized kidneys were immediately fixed in 2.5% glutaraldehyde and then washed in phosphate buffer. After dehydration with alcohol, the samples were embedded in epoxy resin for thin sectioning and electron microscopy. The samples came from two patients homozygous for the Fin-major mutation, two patients who were compound heterozygous for the Fin-major and Fin-minor mutations, and one patient with Fin-major mutation in one chromosome and a missense mutation R743C in the other chromosome (Fig. 1).

Expression of nephrin in NPHS1 kidneys

In situ hybridization for nephrin mRNA was performed as previously described [13]. Briefly, tissue sections were incubated with 1.2×10^6 ³³P-labeled (1000 Ci/mmol; Amersham, Arlington Heights, IL, USA) antisense and sense riboprobes in a total volume of 80 μ L. The probes were synthesized by subcloning a cDNA fragment of 287 bp corresponding to exon 10 in human *NPHS1* gene into pBluescript and antisense and sense RNAs produced using T3 and T7 RNA polymerases, respectively. Paraformaldehyde-fixed cryosections came from two patients homozygous for the Fin-major mutation, two patients homozygous for the Fin-minor mutation, and two patients who were compound heterozygous for these mutations.

Immunofluorescence studies of nephrin in nephrectomized kidneys and in one kidney biopsy were performed in a standard fashion. Paraformaldehyde-fixed cryosections were used. The tissue samples came from four patients homozygous for Fin-major mutation, one patient homozygous for Fin-minor mutation, and three patients who were compound heterozygous for these. One sample came from a child with Fin-major mutation in one chromosome and R743C missense mutation in the other (Fig. 5). Two polyclonal rabbit anti-nephrin antisera were available. One was directed against the extracellular [10] and the

other against the intracellular part of the nephrin molecule (Ruotsalainen et al, manuscript in preparation). Antiserum against ZO-1 came from Zymed Laboratories Inc. (San Francisco, CA, USA).

Western blotting for nephrin was performed on glomerular extracts obtained from four nephrectomized kidneys. Glomerular sieving was performed as previously described [14]. The samples came from two patients homozygous for the Fin-major mutation and two patients with compound heterozygotes for Fin-major and Fin-minor mutations. Ten micrograms of Triton X-100-soluble protein were separated on 4 to 20% gradient gel and transferred into polyvinyl difluoride membrane. Membrane was blotted with monoclonal antibody reacting against the extracellular part of nephrin (Ruotsalainen et al, manuscript in preparation), and mouse IgG was detected with alkaline phosphatase-conjugated anti-mouse IgG (Dako Corporation, Carpinteria, CA, USA) and 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt and p-nitro blue tetrazolium chloride.

Statistics

Statistical analyses were carried out using the Student's *t*-test.

RESULTS

Neonatal and nonrenal features

The majority (38 out of 46, 83%) of NPHS1 children were born prematurely (<38th week) with a birth weight ranging from 1450 to 3480 g. Only two newborns were small for their gestational age. Amniotic fluid was often meconium stained, but a great majority of the neonates did not have pulmonary problems. The index of placental weight/birth weight (ISP) was over 25% in all except one newborn with Fin-major/Fin-major mutations. No

Table 2. Renal findings in NPHS1 patients at the nephrotic stage

Patient genotype	Nephrosis diagnosed at the first week	Proteinuria >10 g/L	Hematuria >100/field	Albumin substitution g/kg/day	Histologic score ^a
Major/major	19/26 ^b	12/15 ^b	15/16 ^b	4.1 (2.8–6.3) ^c (N = 16)	5.0 (0–10) (N = 24)
Major/minor	8/8	3/5	5/5	3.7 (0.9–4.9) (N = 6)	6.9 (3–12) (N = 7)
Minor/minor	4/4	3/4	4/4	3.4 (1.6–4.1) (N = 4)	5.8 (1–10) (N = 4)
Others ^d	3/4	4/5	1/1	3.7 (3.0–4.5) (N = 3)	5.5 (1–12) (N = 4)

^a The number of obsolescent glomeruli, extension of tubular microcysts, interstitial fibrosis and inflammation were graded from nil to 3 and added into a histologic score (maximum score 12) (**Methods** section)

^b Number of patients with the finding/total number of patients with adequate data

^c Results are given as means and range

^d The same as in Table 1

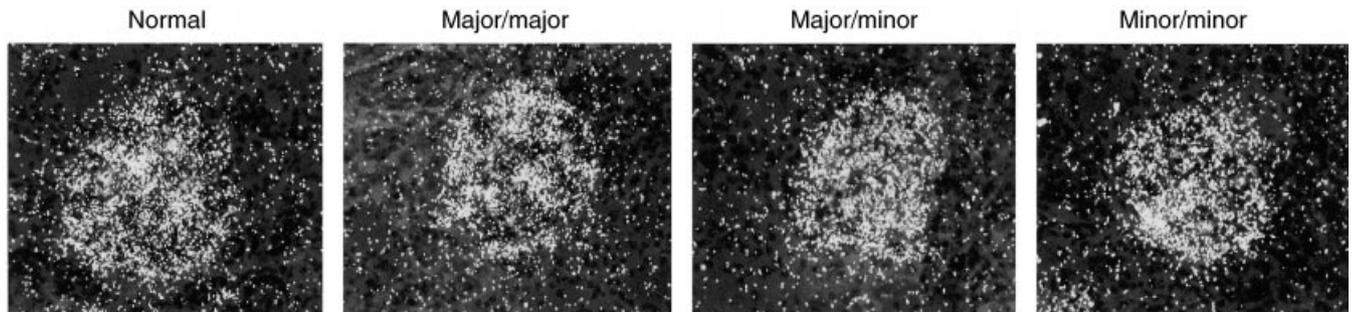


Fig. 2. In situ hybridization for nephrin mRNA in normal and NPHS1 kidneys. A quite normal signal is seen in kidney glomeruli from patients with Fin-major and Fin-minor mutations.

major differences were observed in the neonatal findings between the different genotypes (Table 1).

Infants with NPHS1 did not have any major nonrenal malformations. On the other hand, minor functional disorders in central nervous system and heart were quite common during the nephrotic stage. Most of the children had muscular hypotonia regardless of the mutation (Table 1). EEG was performed to 41 infants at the age of 6 to 12 months. The finding was normal in 28 (68%) cases. Mild or moderate background activity was recorded in nine patients and focal activity in four patients (Table 1). Either CT or magnetic resonance imaging (MRI) or both were performed on 41 patients at the age of 6 to 12 months. Over one third (12 out of 26) of patients homozygous for Fin-major mutation, four out of seven compound heterozygotes, and one Fin-minor homozygote had mild atrophic changes.

During the nephrotic stage, many children had mild cardiac hypertrophy, as shown by x-ray films. Cardiac evaluation, including ultrasound examination, was performed in 42 patients during the nephrotic stage. Minor disorders, including cardiac hypertrophy, mild pulmonary stenosis, and atrial septal defect, were observed, but there was no accumulation of any of these defects in any patient groups (Table 1).

Renal findings

Proteinuria and low serum albumin levels were usually already found in the first samples taken after birth in

patients with different mutations. In 82% of the infants, nephrotic syndrome was diagnosed within the first week after birth (Table 2). The diagnosis was delayed from two weeks to two months in seven infants homozygous for the Fin-major mutation (including two pairs of twins). No difference in the rate of hematuria between patients with different mutations could be found. Also, no difference in the amount of intravenous albumin substitution needed to maintain serum albumin over 15 g/L was detected. All patients had normal creatinine values during the first months. Five patients with Fin-major and Fin-minor mutations received angiotensin-converting enzyme (ACE) inhibitor therapy without a need for a reduction of proteinuria or albumin substitution.

The kidneys were nephrectomized when the infants weighed over 7 kg. In histopathological examination, glomeruli showed mesangial hypercellularity, hyperlobulated capillary tufts, and some scarring. Also, microcystic dilation of both proximal and distal tubuli as well as interstitial fibrosis and inflammation could be observed (Table 2). No major differences were found between different genotypes.

Nephrin and the slit diaphragm in NPHS1 kidneys

In situ hybridization for nephrin mRNA showed quite normal signal in nephrectomized NPHS1 kidneys with Fin-major or Fin-minor mutations (Fig. 2). However, immunofluorescence staining with antibodies reacting against the intracellular and extracellular part of nephrin

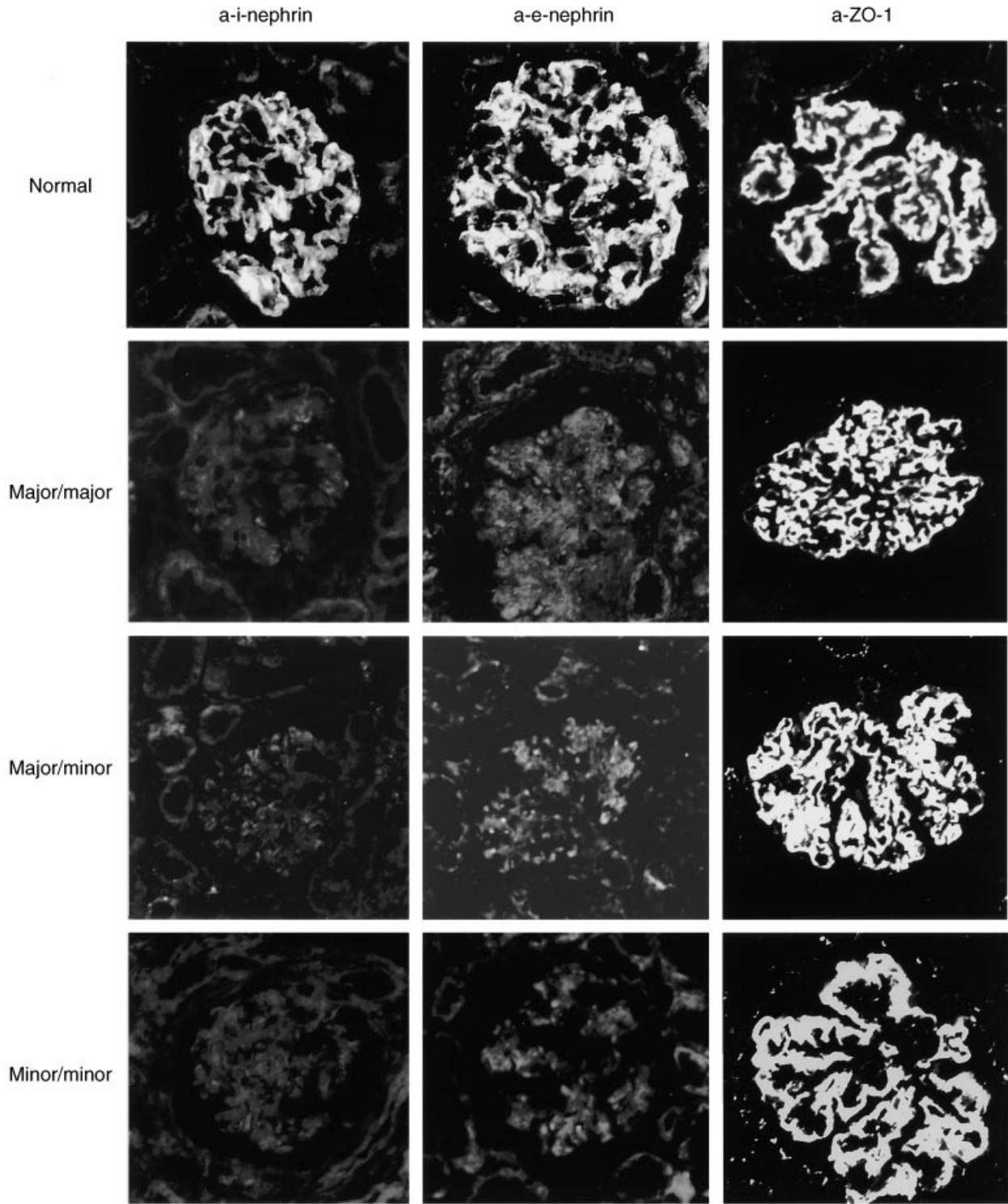


Fig. 3. Immunofluorescence staining for nephrin and ZO-1 in normal and NPHS1 kidneys. No staining of nephrin is seen in NPHS1 glomeruli with different genotypes using polyclonal antibodies against the extracellular part (a-e-nephrin) or intracellular part (a-i-nephrin) of nephrin. The staining pattern of ZO-1 (a-ZO-1) is similar to that of normal kidney in all genotypes.



Fig. 4. Western immunoblotting for nephrin in glomerular extracts from normal and NPHS1 kidneys. No expression of nephrin is seen in glomeruli with Fin-major and Fin-minor mutations, as detected by a monoclonal antibody against the extracellular part of nephrin.

remained negative in kidneys with Fin-major and Fin-minor mutations (Fig. 3). The expression of another slit diaphragm protein, ZO-1, was normal (Fig. 3). In Western blot analysis, a monoclonal anti-nephrin antibody showed no immunoreactive bands in Triton-X extracts of isolated glomeruli from NPHS1 kidneys with Fin-major and Fin-minor mutations (Fig. 4).

Electron microscopy of the glomeruli showed podocyte fusions, as reported previously [15]. Irregular podocyte foot processes were also seen in kidneys with Fin-major and Fin-minor mutations (Fig. 5). Filtration slits between the foot processes were of different sizes. In about a quarter, the slits were extremely narrow (<20 nm), and on the other hand, slits with a width of up to 90 nm were occasionally observed. More importantly, no filamentous image of slit diaphragm was seen in any of the 76 filtration slits analyzed in NPHS1 glomeruli with Fin-major and Fin-minor mutations (Fig. 5). In normal kidneys, the image of slit diaphragm was seen in 52 of the 82 podocytes slit areas (64%) studied. The width of the filtration slit also showed less variation in normal glomeruli and was between 25 and 50 nm in all slit pores studied.

Findings in patients with missense mutations

Figure 1 lists the five patients with missense mutations in at least one gene. Four of these were clinically indistinguishable from children with Fin-major and/or Fin-minor mutations in both genes. Unfortunately, all of these four were born in the early 1990s, and no renal tissue was available for analysis of nephrin expression.

An interesting exception to these findings was a child with Fin-major/R743C mutations (Fig. 6). She was born in 1997, and nephrosis was detected immediately after birth. Proteinuria ranged from 2.8 up to 75 g/L, and ACE inhibitor and indomethacin therapy was started at the age of three months. After two months of therapy, proteinuria decreased to a level of 1 to 3 g/L (Fig. 6A). Renal biopsy was performed. The kidney glomeruli showed

expression of nephrin, and slit diaphragms were evident in electron microscopy (Fig. 6B, C). After taking a kidney biopsy, therapy with prednisone and cyclophosphamide was also tried, but they had no effect on the residual proteinuria. The child is now 2.5 years old with only mild proteinuria (<1 g/L).

DISCUSSION

This study shows that the two most common mutations of the *NPHS1* gene, Fin-major and Fin-minor, both lead to the severe form of congenital nephrosis. The clinical features include prematurity, large placenta, heavy proteinuria, and full-blown nephrosis soon after birth. Also, these patients do not react to ACE inhibitors or indomethacin.

As the Fin-major mutation results in a nephrin molecule of only 90 amino acid residues [9], it seemed probable that Fin-major homozygotes would not show immunoreactivity to nephrin. Indeed, the Fin-major mutation leads to a complete absence of the protein. In contrast, Fin-minor mutation only leads to the loss of a part (132 terminal amino acids) of the intracellular domain of nephrin [9]. Therefore, the protein, although multifunctional, could be expected to be expressed and present on the podocyte plasma membrane. This was, however, not the case, as we saw no immunoreactivity of nephrin in immunofluorescence staining of the kidney sections or immunoblotting of glomerular extracts. The finding that the Fin-minor mutation leads to an absence of nephrin on the podocyte surface was unexpected.

Nephrin has been localized to the slit diaphragm region of glomeruli [10]. Therefore, it was of interest to see how the lack of nephrin in kidneys with Fin-major and Fin-minor mutations affects the ultrastructure of the glomerular filtration barrier. As reported previously [15], an irregular pattern and fusion of podocyte foot processes was seen in electron microscopy. In addition, quite normal-looking podocyte slits were observed. However, no slit diaphragms were present in these slits. This supports the idea that nephrin is an important component of slit diaphragm and plays a crucial role for the glomerular filtration barrier.

The width of the slit pore in NPHS1 glomeruli showed greater variation than in normal kidneys. Narrow slits (<20 nm) were present in NPHS1 kidneys but not in normal glomeruli. Since the structure of the slit diaphragm is still largely unknown, the molecular basis for this phenomenon remains to be seen. The zonula occludens-1-antigen (ZO-1) is a cytosolic component of the slit diaphragm region in human kidney [16], and recently, P-cadherin was localized to the this area [17]. The podocyte surface is also rich of negatively charged glycoproteins, which have an impact on the foot process interactions. The role of these components in the ultra-

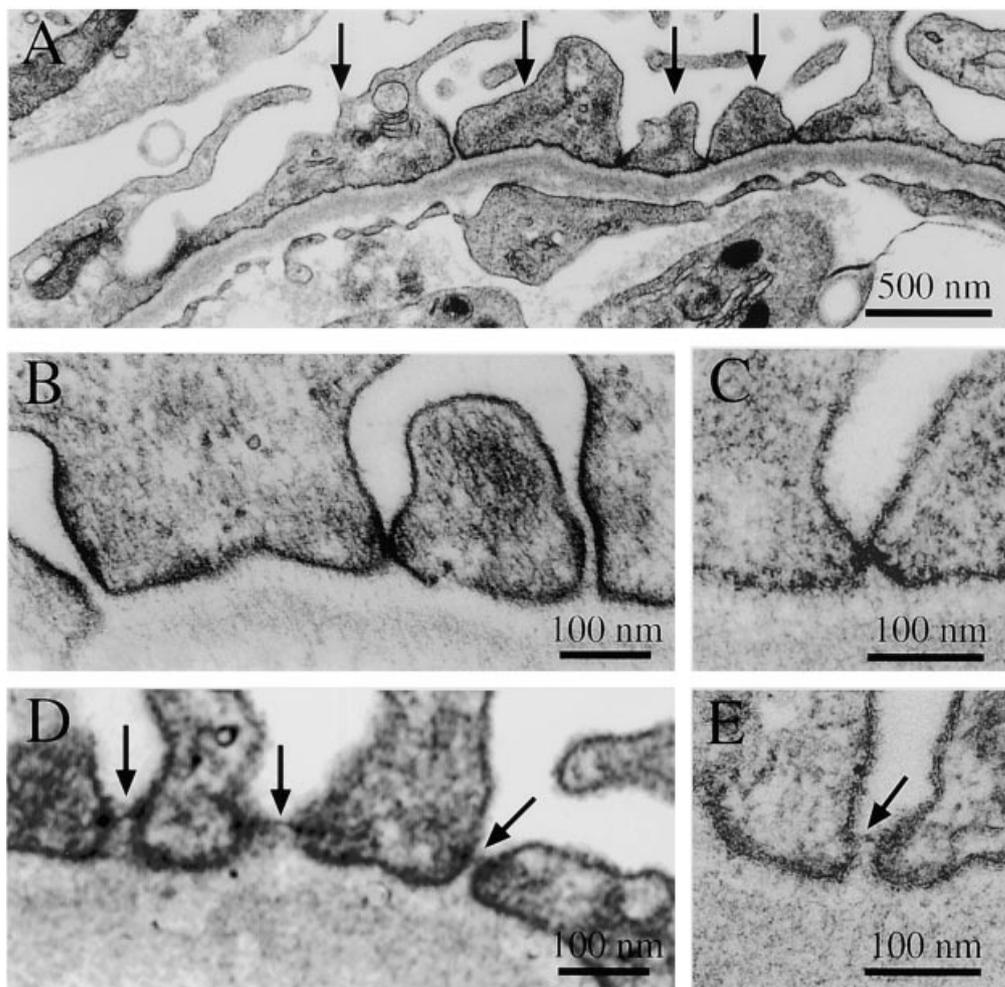


Fig. 5. Electron microscopy of *NPHS1* and control kidneys. (A) The capillary wall in Fin-major homozygote showing podocyte foot processes (arrows) of various size. (B) Filtration slits of various size in the *NPHS1* kidney of a Fin-major/Fin-minor genotype. No slit diaphragms are seen. (C) A narrow filtration slit in a *NPHS1* kidney of Fin-major homozygote. (D and E) Podocyte filtration slits in normal human kidney. The slit diaphragms are seen as filamentous images (arrows).

structural organization of the podocyte slit in normal and *NPHS1* glomerulus is unclear.

Interestingly, the expression of ZO-1 was normal in *NPHS1* kidneys, indicating that the lack of nephrin does not affect the expression of ZO-1. This is different from that seen in rats. Injection of antibodies against a slit diaphragm antigen, p51, induces nephrosis in rats and leads to progressive decline in stainable ZO-1 [18]. It was recently shown that p51 antigen is an epitope of nephrin molecule [19], suggesting that nephrin and ZO-1 in this model are linked together.

Recently, Holthöfer et al described the expression of nephrin in normal and *NPHS1* kidneys using two polyclonal antibodies against nephrin [20]. In immunofluorescence nephrin was not detected in *NPHS1* kidneys, but in immunoelectron microscopy, they noticed some nephrin-specific gold particles at the flattened apical surface of podocytes and rarely at the intercellular junc-

tional areas. They did not correlate these staining patterns to the *NPHS1* genotype, and the significance of their findings remains unclear. In our study, the negative immunofluorescence staining and immunoblotting of the glomerular extracts as well as the lack of slit diaphragm in kidneys with Fin-major and Fin-minor mutations strongly indicate that both mutations lead to the total lack of functional nephrin in these patients.

Based on the immunohistologic and microscopic findings, it is not surprising that the clinical features of children with Fin-major and Fin-minor mutations were similar. Both mutations lead to full-blown nephrosis soon after the birth, and large amounts of albumin were needed to substitute the protein loss. Also, therapy with agents reducing the glomerular filtration pressure were not successful in diminishing the proteinuria. Previously, non-Finnish CNS patients responding to ACE inhibitors and indomethacin have been published [21, 22].

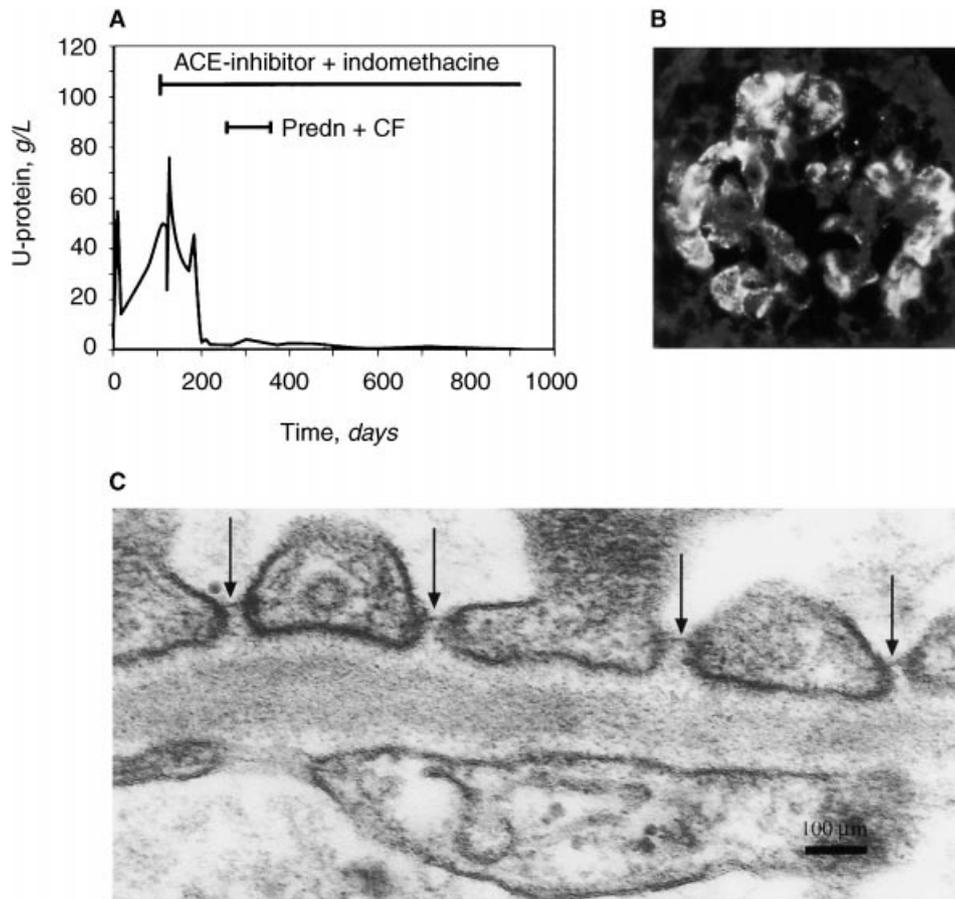


Fig. 6. Urinary protein excretion and renal findings in *NPHS1* patient with Fin-major/R743C mutations. (A) Effect of antiproteinuric therapy on urinary protein excretion. Enalapril and indomethacin were started at the age of three months. The dosing was gradually increased, and two months later (indomethacin 2.2 mg/kg/day and enalapril 1.3 mg/kg/day), a reduction in urinary protein from about 30 g/L to 1 to 3 g/L was observed. After this, prednisone and cyclophosphamide were given for four months and six weeks, respectively, to decrease further the protein excretion. No clear response was observed with either of the drugs. (B) Kidney biopsy was taken at the age of five months (after the decrease of proteinuria), and in immunofluorescence staining, nephrin was expressed normally in the glomeruli. (C) In electron microscopy, fusion of foot processes as well as quite normal-looking foot processes could be seen. Filamentous images of slit diaphragms looked normal between the foot processes (arrows).

Our patient material, however, included one important exception: a child with a Fin-major mutation in one gene and a missense mutation (a change of arginine-743 to cysteine in the extracellular Ig-5 domain) in the other one. Her nephrosis was detected immediately after birth, and proteinuria up to 75 g/L was measured during the first months. However, she responded to enalapril and indomethacin therapy and has avoided renal transplantation. The obvious explanation came from the microscopic studies. Her kidneys expressed nephrin, and slit diaphragms were present between the podocyte foot processes. Thus, although the missense mutation in the *NPHS1* gene led to a “functional” disorder in her glomerular filtration barrier, the pharmacological reduction of the glomerular filtration pressure was enough to reduce the protein leakage dramatically.

Recently, over 30 mutations have been detected in non-Finnish patients with CNS [11]. It is clear that these mutations may lead to the severe, therapy-resistant form

(so-called Finnish type) or to a less severe clinical picture of CNS. Based on the present data, a renal biopsy on infants with congenital nephrosis who are not Fin-major or Fin-minor homozygotes or compound heterozygotes may be indicated. If the kidneys express nephrin and electron microscopy reveals the presence of slit diaphragms, a response to an ACE inhibitor and indomethacin might be expected. On the other hand, if immunofluorescence staining for nephrin is negative, the chances for a response are small, and renal transplantation seems to be the only option.

The expression of nephrin in nonrenal tissues is not completely known. Northern hybridization did not reveal nephrin-RNA in fetal placenta, liver, brain, lung, muscle, or pancreas [9]. Recent findings, however, have shown that mRNA for nephrin is present in cells of the neural tube in developing mouse embryos (Putala et al, manuscript submitted for publication). Clinically, children with *NPHS1* do not have major defects in other

organs than the kidney [1]. However, minor neurological problems during the nephrotic stage can be observed in these children. Many patients are hypotonic, and their psychomotoric development is slow. Later on, vascular changes and various degrees of brain atrophy are noticed in these patients. Although one could argue that some of these symptoms could be related to an absence of nephrin in the central nervous system, it seems more probable that they are due to the heavy proteinuria and protein deficiency as such. This is supported by the fact that after nephrectomy, muscular hypotonia and neurological status improve dramatically [23]. Those children who have had severe complications such as deep vein thrombosis or infarcts during nephrosis, of course, will suffer from permanent handicaps (Qvist et al, manuscript in preparation).

Hypoproteinemia and its effects on hemodynamics most probably also are responsible for the cardiac hypertrophy seen in many NPHS1 infants during the nephrotic stage. After nephrectomy, the cardiac findings gradually normalize, and no permanent heart defects are seen in these children. The explanation for the large placenta still remains open. *In situ* hybridization for nephrin mRNA as well as immunohistochemical stainings (Pattrakka et al, unpublished observations) clearly indicate that nephrin is not expressed in the placenta.

We conclude that *NPHS1* mutations causing an absence of nephrin and podocyte slit diaphragms are responsible for a severe, therapy-resistant form of NPHS1 (the Finnish type), while patients with *NPHS1* mutations causing only partially defective nephrin may still have slit diaphragms and respond to therapy.

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