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Nephrotic Syndrome in the First Year of Life: Two Thirds of Cases Are Caused by Mutations in 4 Genes (*NPHS1*, *NPHS2*, *WT1*, and *LAMB2*)

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ABSTRACT

OBJECTIVES. Mutations in each of the *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* genes have been implicated in nephrotic syndrome, manifesting in the first year of life. The relative frequency of causative mutations in these genes in children with nephrotic syndrome manifesting in the first year of life is unknown. Therefore, we analyzed all 4 of the genes jointly in a large European cohort of 89 children from 80 families with nephrotic syndrome manifesting in the first year of life and characterized genotype/phenotype correlations.

METHODS. We performed direct exon sequencing of *NPHS1*, *NPHS2*, and the relevant exons 8 and 9 of *WT1*, whereas the *LAMB2* gene was screened by enzymatic mismatches cleavage.

RESULTS. We detected disease-causing mutations in 66.3% (53 of 80) families (*NPHS1*, *NPHS2*, *WT1*, and *LAMB2*: 22.5%, 37.5%, 3.8%, and 2.5%, respectively). As many as 84.8% of families with congenital onset (0–3 months) and 44.1% with infantile onset (4–12 months) of nephrotic syndrome were explained by mutations. *NPHS2* mutations were the most frequent cause of nephrotic syndrome among both families with congenital nephrotic syndrome (39.1%) and infantile nephrotic syndrome (35.3%), whereas *NPHS1* mutations were solely found in patients with congenital onset. Of 45 children in whom steroid treatment was attempted, only 1 patient achieved a lasting response. Of these 45 treated children, 28 had causative mutations, and none of the 28 responded to treatment.

CONCLUSIONS. First, two thirds of nephrotic syndrome manifesting in the first year of life can be explained by mutations in 4 genes only (*NPHS1*, *NPHS2*, *WT1*, or *LAMB2*). Second, *NPHS1* mutations occur in congenital nephrotic syndrome only. Third, infants with causative mutations in any of the 4 genes do not respond to steroid treatment; therefore, unnecessary treatment attempts can be avoided.

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Key Words
nephrotic syndrome, LAMB2, NPHS1, NPHS2, WT1

Abbreviations
NS—nephrotic syndrome
NSFL—nephrotic syndrome manifesting in the first year of life
CNS—congenital nephrotic syndrome
INS—infantile nephrotic syndrome
OMIM—Online Mendelian Inheritance in Man
ESRD—end-stage renal disease

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Fourth, there are most likely additional unknown genes mutated in early-onset nephrotic syndrome.

NEPHROTIC SYNDROME (NS), the association of gross proteinuria, hypoalbuminaemia, edema, and hyperlipidemia, is often a life-threatening condition when manifesting as NS in the first year of life (NSFL). Whereas standardized treatment protocols are widely used for older children,[1,2] in whom a response rate to steroid treatment of ≤80% can be observed, clinical decision-making in children with NSFL remains challenging. Descriptively, NSFL has been classified as congenital NS (CNS), manifesting in utero or during the first 3 months of life, or infantile NS (INS), with onset between 4 months and 1 year of age.

Over the last few years, inherited impairments of the glomerular filtration barrier have been identified as important causes of NS. The glomerular filtration barrier separates the bloodstream from the urinary space and consists of 3 layers, fenestrated endothelium, glomerular basement membrane, and podocyte foot processes. The interdigitating podocyte foot processes are connected by the slit-diaphragm. The protein nephrin, encoded by the *NPHS1* gene, represents a major component of this slit-diaphragm and connects foot processes in a zipper-like fashion.[3] Mutations of *NPHS1* were identified by Kestila et al[4] as the cause of CNS of the Finnish type (Online Mendelian Inheritance in Man [OMIM] No. 256300). They result in a disruption of the glomerular filtration barrier and consecutive massive protein loss. Detailed studies on *NPHS1* were undertaken, and 2 mutations Fin_{major} (L41fsX90) and Fin_{minor} (R1109X) were identified as the predominant cause of CNS in the Finnish population.[4] Mutations in the *NPHS2* gene, encoding podocin, were then identified by Boute et al[5] as a cause of autosomal recessive steroid-resistant NS (OMIM No. 600995) among older children. Podocin represents an essential component of the slit-diaphragm and a close interactor of nephrin. Intracellular retainment of podocin mutants[6] and consecutively altered trafficking of nephrin have been demonstrated.[7] Mutations of podocin also result in a dysfunction of the glomerular filtration barrier. The preceding studies demonstrated that children with *NPHS2* mutations may manifest as NSFL,[8–11] but no systematic evaluation focusing on *NPHS2* in infants has been undertaken. Furthermore, we recently reported dominant de novo mutations in exons 8 and 9 of the *WT1* gene (encoding the transcription factor Wilms tumor suppressor gene 1) as a cause of isolated steroid-resistant NS in infants and children.[12] Transcriptional activation of *NPHS1* and upregulation of *NPHS1* mRNA by *WT1* have been shown.[13,14] These findings explain why mutations in *WT1* result in NS. In addition, *WT1* mutations have been detected in patients with Wilms tumor (OMIM No. 194070),[15,16] Denys-Drash syndrome (OMIM No. 194080),[17] and Frasier syndrome (OMIM No. 136680).[18] Finally, laminin-β2 as a component of the glomerular basement membrane is crucial for podocyte foot process architecture and stability but is also found in other organs, such as the eye.[19] Missense mutations of the *LAMB2* present with a spectrum of symptoms reaching from isolated early onset NS[20] to intermediate phenotypes, whereas patients with truncating *LAMB2* mutations present with the full syndromic phenotype of Pierson syndrome with NS, diffuse mesangial sclerosis, distinct eye anomalies, and mental retardation (OMIM No. 609049).[19]

Mutations in the genes *TRPC6*,[21] *ACTN4*,[22] and *CD2AP*[23] cause adult-onset NS. They follow a dominant pattern of inheritance and lead to focal segmental glomerulosclerosis. Because they have not been reported in children with NSFL, they were not included in this study.

Mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* in NSFL have been described in small patient cohorts only. These studies mainly focused on 1 of these genes respectively. No reliable data on the relative frequencies of mutations in these genes among children with NSFL are available. We, therefore, examined within a large cohort of 975 children with NS ascertained over 10 years, the subgroup of 89 European infants with NSFL for mutations in *NPHS1*, *NPHS2*, *WT1*, and *LAMB2*. Here we demonstrate that, in two thirds of children with NSFL, a genetic cause can be identified in 1 of these 4 genes and that children with these mutations do not respond to steroid treatment.

PATIENTS AND METHODS

Patient and Data Recruitment

In this worldwide study, DNA samples and clinical data from 975 children with NS have been ascertained between 1996 and 2005 (www.renalgenes.org/nephrotic_syndrome.html). Patient recruitment after informed consent has been described by Ruf et al.[8] The focus of this study was on a subgroup of children manifesting with NSFL. Patients were enrolled by pediatric nephrologists from specialized centers only. Diagnosis of NS and, where applicable, response to steroid treatment were classified according to published criteria.[1] Evaluation of clinical characteristics was based on a standard questionnaire described previously (www.renalgenes.org/nephrotic_syndrome.html).[24] Parameters characterized in this analysis were: age of onset, ethnic origin, histology findings on kidney biopsies or nephrectomy, response to standard steroid treatment, and clinical course from first presentation to last clinical examination.

Between 1996 and 2005, 107 children suffering from either sporadic or familial NSFL were enrolled in the

study. To better define our group by ethnic origin, we excluded 18 non-European children from Asia and North America. The remaining 89 patients were of non-Scandinavian European descent, including 25 patients of Turkish descent. The 89 European patients studied were from 80 different families and included 71 families with 1 affected child and 9 families with 2 affected children with NSFL (Table 1). Parental consanguinity was documented for 4 sibling pairs and 15 children with sporadic NSFL. Fifty-four children from 46 families were classified as having CNS with first documented presentation in utero or within 90 days from birth (13 patients of Turkish descent). Thirty-five children from 34 families were classified as having INS with first documented presentation between 91 and 365 days (12 patients of Turkish descent). Partial data on 31 patients from 25 families have been published previously.[8,9,12,20] In this work, we will refer to the number of patients studied when assessing clinical data and to the number of families studied when assessing genetic data, because affected siblings may follow a different clinical course but should bear the same mutations.

Mutation Analysis by Direct Sequencing for *NPHS1*, *NPHS2*, and *WT1*

Genomic DNA was directly isolated from blood samples using the Puregene DNA purification kit (Gentra, Minneapolis, MN) following the manufacturer's guidelines. Mutation analysis was performed by direct sequencing of all 29 exons of *NPHS1*, all 8 exons of *NPHS2*, and exons 8 and 9 of *WT1*. *WT1* analysis was limited to exons 8 and 9, because mutations of this gene accounting for isolated NS have only been reported in these 2 exons.[12] All of the exons were amplified by PCR and sequenced as described previously.[25] For sequence analysis, the software SEQUENCHER (Gene Codes, Ann Arbor, MI) was used. For all of the detected mutations and new sequence variants, sequencing of both strands was performed. Segregation of these changes was confirmed by direct sequencing of parental DNA samples where available. The absence of previously unreported mutations was shown in 160 control chromosomes from healthy individuals of matched ethnic origin.

CEL I Nuclease Assay for *LAMB2* Screening

To screen all 32 exons of the *LAMB2* gene in 89 patients, the CEL I nuclease assay was used.[26] This assay has a sensitivity of >92% in our hands (E. Otto, PhD, verbal communication, 2006). CEL I nuclease was extracted from celery plants as previously described by Till et al.[26] To detect homozygous mutations in addition to heterozygous ones, DNA strands of affected and healthy control individuals were mixed after exon PCR. Samples were denatured at 95°C and cooled to allow heteroduplexes to form. After incubation of heteroduplexes with CEL I nuclease for 5 minutes, the enzymatic cleavage at sites of DNA mismatches was terminated by the addition of 0.5 m of EDTA and immediate incubation on ice. When products were run on a 1% agarose gel, the presence of ≥2 bands indicated that cleavage had occurred at the site of a mismatch. Such aberrant samples were then chosen for mutation analysis by direct DNA sequencing as described above.

RESULTS

To evaluate the frequency of mutations in *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* in patients with NSFL, we screened 89 affected European children from 80 families by direct sequencing of all exons of *NPHS1*, *NPHS2*, and the relevant exons 8 and 9 of *WT1*. Using an enzymatic CEL I nuclease cleavage assay, all of the exons of *LAMB2* were screened. We then retrospectively analyzed the clinical outcome of these children.

TABLE 1 **Frequency of Disease-Causing Mutations in *NPHS1*, *NPHS2*, *LAMB2*, and *WT1* in 80 Families With NSFL**

Age of Onset	No. of Families	Mutations					No Mutations, *n* (%)
		Total, *n* (%)	*NPHS1*, *n* (%)	*NPHS2*, *n* (%)	*WT1*, *n* (%)	*LAMB2*, *n* (%)	
First year of life (CNS + INS)							
All	80	53 (66.3)	18 (22.5)	30 (37.5)	3 (3.8)	2 (2.5)	27 (33.7)
CEU	57	43 (75.5)	12 (21.0)	27 (47.4)	3 (5.3)	1 (1.8)	14 (24.5)
Turkish	23	10 (43.5)	6 (26.1)	3 (13.1)	0 (0.0)	1 (4.3)	13 (56.5)
CNS							
All	46	39 (84.8)	18 (39.1)	18 (39.1)	1 (2.2)	2 (4.4)	7 (15.2)
CEU	35	32 (91.4)	12 (34.3)	18 (51.4)	1 (2.85)	1 (2.85)	3 (8.6)
Turkish	11	7 (63.6)	6 (54.5)	0 (0.0)	0 (0.0)	1 (9.1)	4 (36.4)
INS							
All	34	15 (44.1)	0 (0.0)	12 (35.3)	2 (5.9)	1 (2.9)	19 (58.9)
CEU	22	12 (54.5)	0 (0.0)	9 (40.9)	2 (9.0)	1 (4.5)	10 (45.5)
Turkish	12	3 (25.0)	0 (0.0)	3 (25.0)	0 (0.0)	0 (0.0)	9 (75.0)

CEU indicates central European.

Frequencies of Mutations

Frequencies of detected mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* in 80 European families with NSFL are summarized in Table 1. The correlation of detected mutations with exact age at first clinical presentation for 89 affected individuals is shown in Fig 1.

Mutation analysis of *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* revealed disease-causing mutations in 66.3% of all of the families with NSFL (Table 1), thereby identifying the molecular cause of NSFL in 53 of 80 of all studied families. Disease-causing mutations in *NPHS1* were found in 22.5% (18 of 80) of all of the families. Mutations in *NPHS2* were identified in 37.5% (30 of 80) of all of the families as the most common cause of NSFL and accounted for 56.6% (30 of 53) of all of the causative mutations. Dominant mutations in *WT1* were found in 3.8% (3 of 80), and recessive mutations in *LAMB2* in 2.5% (2 of 80) of families. Among 33.7% (27 of 80) of our families in whom NSFL was not explained by mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2*, 4 children were carriers of a single heterozygous *NPHS1* mutation, which cannot be considered causative in itself. All 9 of the sibling pairs in this study carried mutations in *NPHS1* (3), *NPHS2* (4), or *LAMB2* (2). Seventeen of these 18 children with "familial" NSFL manifested within the first 3 months of life and 1 child at 4 months of age. The mutation rate of families with >1 affected child was, thus, 100% (9 of 9); the rate among families with 1 affected child only was 62% (45 of 71).

The frequencies of mutations in *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* differed between children with congenital and infantile onset (Table 1). In CNS (onset 0–3 months), mutations were found in 1 of the 4 genes in 84.8% (39 of 46) of all families. The distribution was *NPHS1*, *NPHS2*, *WT1*, and *LAMB2*: 39.1% (18 of 46), 39.1% (18 of 46), 2.2% (1 of 46), and 4.4% (2 of 46). In 15.2% (7 of 46) of families with CNS in whom the disease could not be explained by mutations in these 4

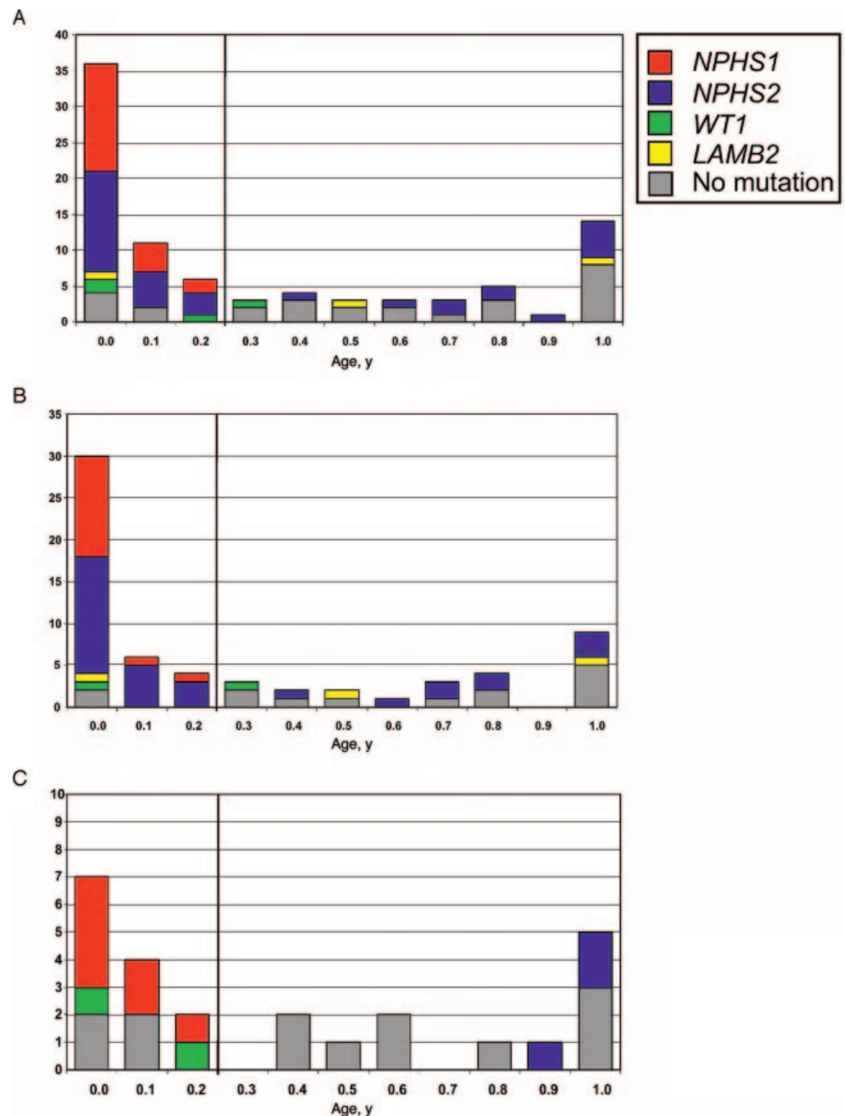

FIGURE 1
Age at diagnosis of NS in correlation to detected pathogenic mutation in *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* in 89 children from 80 families with NSFL. A, 89 central European and Turkish children: 60.7% (54 of 89) of individuals manifested within the first 3 months of life (CNS). Of these, 87.0% (47 of 54) carried disease-causing mutations. Note that *NPHS1* mutations were detected only in CNS. B, Subgroup of 64 central European children: 64.1% (41 of 64) of the children manifested with CNS, and the majority carried mutations in *NPHS2* (22 of 41 [53.7%]). C, Subgroup of 25 Turkish children: 52.0% (13 of 25) of the children manifested with CNS, and none of these children carried mutations in *NPHS2*. In 52.0% (13 of 25) Turkish children, no mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* were found.

genes, we detected only 1 heterozygous *NPHS1* mutation in 3 families. In *NPHS1*, all of the patients with disease-causing mutations presented as CNS, that is, within the first 3 months of life. Manifestation of children with *NPHS1* mutations was, thus, limited to congenital onset. In INS (onset 4–12 months), mutations were found in 44.1% (15 of 34) of families. The distribution was *NPHS1*, *NPHS2*, *WT1*, and *LAMB2*: 0.0% (0 of 34), 35.2% (12 of 34), 5.9% (2 of 34), and 2.9% (1 of 34; Table 1). The percentage of patients in whom the disease was explained by mutations in these 4 genes, thus, decreased with increasing age at first presentation. Mutations in *LAMB2* and *WT1* were a rare cause of NSFL between both groups, CNS and INS, and accounted together for NSFL in only 6.3% (5 of 80) of all families.

The frequencies of mutations in *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* were different between children of central European and of Turkish descent (Table 1 and Fig 1). In families of central European descent, mutations were found in 75.5% (43 of 57) of families. The distribution was, respectively, *NPHS1*, *NPHS2*, *WT1*, and *LAMB2*: 21.0% (12 of 57), 47.4% (27 of 57), 5.3% (3 of 57), and 1.8% (1 of 57; Fig 1A). Mutations were found in 43.5% (10 of 23) of families of Turkish descent. The distribution was, respectively, *NPHS1*, *NPHS2*, *WT1*, and *LAMB2*: 26.1% (6 of 23), 13.1% (3 of 23), 0.0% (0 of 23), and 4.3% (1 of 23; Fig 1B). Although in families of central European descent, mutations in *NPHS2* represented the most frequent cause of CNS (18 of 35 [51.4%]), no mutations of this gene were seen in 13 families of Turkish descent with CNS (Fig 1).

Spectrum of Mutations

An exact reference of all of the detected mutations is given in the Appendix.

NPHS1

A spectrum of 20 different homozygous or compound heterozygous mutations was found in *NPHS1*. One single Fin_{major} (F1017) mutation, but no Fin_{minor} mutations, were seen. In 3 patients presenting with CNS and a fourth patient presenting at 3.5 months of age, only a single heterozygous *NPHS1* mutation was detected. Four patients with compound heterozygous disease-causing *NPHS1* mutations carried the *NPHS2* sequence variant R229Q, in addition, which is considered a polymorphism.

NPHS2

A founder mutation R138Q has been described by Boute et al.[5] We confirm R138Q as the most frequent mutation as follows. In 30 families (18 with CNS and 12 with INS) with *NPHS2* mutations, the founder mutation R138Q was detected homozygously in 12 families and compound heterozygously in 8 families. In CNS, all of the patients with *NPHS2* mutations carried ≥1 loss of function mutation or R138Q, with the exception of patient F876. In INS, all of the patients with *NPHS2* mutations, except for patient F1336, showed either a loss of function mutation or the founder mutation R138Q. The spectrum of detected mutations in *NPHS2* was, therefore, similar for patients with CNS and INS.

WT1

The 3 mutations detected in *WT1* (R366H [A651], D396N [A794], and R395P [A1021]) were de novo changes of exons 8 or 9 manifesting as a dominant disease in female patients.[12,16,27,28]

LAMB2

The disease-causing mutations detected in the *LAMB2* gene of families F1012 (R246Q) and F1234 (C231R and L1393F) were missense mutations as further described by Hasselbacher et al.[20]

Genotype Versus Treatment Response

Steroid treatment was attempted in 50.6% (45 of 89), not given in 30.3% (27 of 89), and not reported in 19.1% (17 of 89) of children. Detailed clinical data of all 89 individuals with NSFL are provided in the Appendix.

Among children with CNS, steroids were administered less frequently (21 of 54 [38.9%]) than among patients with INS (24 of 35 [68.6%]). Only 6 treated children showed an initial response to steroids. Five of these children showed no mutations in the 4 genes, and 1 (F1313) carried a single heterozygous *NPHS1* mutation, P264R, which also does not explain the phenotype. Among the 45 treated children, 5 of 6 initially responsive children relapsed early, whereas 1 child (A90) of Turkish descent achieved a continuous remission through the steroid treatment. This child manifested at 9 months of age, and carried only 1 single *NPHS2* R229Q polymorphism. Therefore, 97.8% (44 of 45) of treated children did not achieve a lasting response under steroids, whereas the only child to respond to steroid treatment did not carry disease-causing mutations. In 62.2% (28 of 45) of treated children, disease-causing mutations were detected; none of these children responded to steroid treatment.

Genotype Versus Onset of End-Stage Renal Disease

Evaluation of the clinical follow-up was possible for 88.6% (79 of 89) of patients. At last examination, 46.8% (37 of 79) of children had not reached end-stage renal disease (ESRD), 6.3% (5 of 79) were in ESRD and had not received a kidney transplant, 27.8% (22 of 79) had received a kidney transplant and did not experience transplant failure, 7.6% (6 of 79) had suffered from kidney-transplant failure, and 11.4% (9 of 79) were deceased.

Progression to ESRD was more rapid in children with *NPHS1* mutations than in children with *NPHS2* muta-

tions (P = .04). Nine children with *NPHS1* mutations in ESRD had progressed to ESRD within a mean interval of 4.6 years (range: 1.8–9.3 years) from diagnosis, whereas the equivalent group of 16 children with *NPHS2* mutations had developed ESRD after a mean of 6.6 years (range: 2.8–10.0 years). Four of the 19 children with *NPHS1* mutations had died of their disease, whereas all 27 children with *NPHS2* mutations were alive. Two of 17 children with *NPHS2* mutations and kidney transplant (F1221 and A674) showed recurrence of focal segmental glomerulosclerosis in the transplant, and 2 had a rejection of their transplant (A126 and F1030). In 1 patient with disease-causing *NPHS1* mutations (F475 II-1), no function was obtained, and in 1 patient with a single *NPHS1* mutation (A693), the transplant was rejected.

DISCUSSION

We present data on 89 children from 80 different families of European descent manifesting with NS in the first year of life. This is the largest cohort of children with this diagnosis published to date who underwent a combined molecular genetic evaluation of *NPHS1* and *NPHS2* and the first to include *WT1* and *LAMB2*.

We found a high rate of mutations clarifying the molecular pathogenesis in 66.3% of all 80 families with NSFL and in 84.8% of all families with CNS by screening the 4 known genes *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* only. These data show that NSFL is, to a large extent, a monogenic disease, in which genetic testing should be considered as an early diagnostic step. These findings have additional clinical relevance, because data on the relative frequency of genetically caused NSFL has been missing, and data on the prevalence of steroid responsiveness among children with NSFL is sparse. The efficacy of steroid treatment in infants with NS is considered to be low.[29,30] However, the documented initiation of steroid treatment in more than half of all patients (45 of 89) reported here illustrates that this treatment remains in clinical practice in children with NSFL.

For 45 children in this study who were treated with steroids, an initial response to treatment was reported in 6 children only. Just 1 of these 6 initially responsive children (A90) achieved a remission but did not carry a disease-causing mutation in 1 of the 4 analyzed genes. In 28 (62.2%) of 45 treated children, we found disease-causing mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2*. None of the children who were treated with steroids and who carried a mutation showed a response. We, therefore, conclude that mutation analysis of *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* is warranted in children with NSFL. If DNA analysis reveals pathogenic mutations, the children should be spared the adverse effects of unwarranted steroid treatment attempts.

Our data show that children with mutations in *NPHS1* always manifest within the first 3 months of life. Only small numbers of cases with *NPHS2* mutations and manifestation during these first 3 months of life have been described.[8,10] Our findings now identify *NPHS2* mutations as a frequent cause of CNS among patients of central European descent (51.4%). This finding emphasizes the necessity to include in the mutation analysis of CNS not only *NPHS1* but also *NPHS2.* If no mutations are found in these 2 genes, the screening of *WT1* and *LAMB2* may be indicated, because mutations were found in 3.8% and 2.5% of CNS, respectively.

The relative frequency of mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* in patients with NSFL should be seen in the context of the patients' ethnic origin. Turkish children in our cohort had a higher percentage of absence of mutations in any of the 4 genes (56.5%) compared with central European children (24.5%). In contrast to central European children, no *NPHS2* mutations were found in Turkish children with CNS. The spectrum of mutations in NSFL has been reported to vary by ethnicity for *NPHS1* and *NPHS2*. Mutations in *NPHS1* show a high incidence in the Finnish population and are almost the exclusive cause of CNS there.[4,31] Two truncating mutations, Fin_{major} and Fin_{minor}, of *NPHS1* are predominantly seen in the Finnish population.[4] Outside Finland, however, this Finnish-type CNS is less frequent, and a diversity of different mutations are found in *NPHS1*.[32] Both findings are further supported by our data on mutations, so that further studies will be necessary to evaluate the frequency of mutations in these genes in NSFL among children of different ethnic origin.

Our results reveal a specific spectrum of "severe" *NPHS2* mutations resulting in NSFL. Twenty-eight (93.3%) of 30 families with *NPHS2* mutations carried $\geq$1 truncating mutation or the founder mutation R138Q, a finding also supported by data from Weber et al.[10] Published functional data may provide the first answers as to why these specific alleles cause NS with early onset. It was shown that the *NPHS2* variant R138Q, which we frequently detected in our patients, is retained in the endoplasmatic reticulum disrupting the targeting of *NPHS1* to lipid raft microdomains.[7,33,34] Based on these experimental findings, Nishibori et al[34] speculated that this altered nephrin positioning resulting from the R138Q mutation in *NPHS2* could resemble CNS of the Finnish type not only pathophysiologically but also clinically.

CONCLUSIONS

Based on our findings, we draw the following conclusions: (1) NSFL is, to a large extent, a monogenic disease, and two thirds of NSFL can be explained by mutations in these 4 genes (*NPHS1*, *NPHS2*, *WT1*, or *LAMB2*) only; (2) children with *NPHS1* mutations manifest as CNS only, whereas mutations in *NPHS2* are an additional frequent cause of CNS among central European children; (3) infants with causative mutations in any of the 4 genes do not respond to steroid treatment, and, therefore, unnec-

essary treatment attempts can be avoided; and (4) the identification of additional genes as mutated in NSFL can be anticipated.

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REFERENCES

1. Arbeitsgemeinschaft für Paediatrische Nephrologie. Short versus standard prednisone therapy for initial treatment of idiopathic nephrotic syndrome in children. *Lancet.* 1988;1(8582): 380–383
2. Tune BM, Mendoza SA. Treatment of the idiopathic nephrotic syndrome: regimens and outcomes in children and adults. *J Am Soc Nephrol.* 1997;8:824–832
3. Ruotsalainen V, Ljungberg P, Wartiovaara J, et al. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. *Proc Nat Acad Sci USA.* 1999;96:7962–7967
4. Kestila M, Lenkkeri U, Mannikko M, et al. Positionally cloned gene for a novel glomerular protein–nephrin–is mutated in congenital nephrotic syndrome. *Mol Cell.* 1998;1:575–582
5. Boute N, Gribouval O, Roselli S, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet.* 2000;24: 349–354
6. Roselli S, Moutkine I, Gribouval O, Benmerah A, Antignac C. Plasma membrane targeting of podocin through the classical exocytic pathway: effect of *NPHS2* mutations. *Traffic.* 2004;5: 37–44
7. Huber TB, Simons M, Hartleben B, et al. Molecular basis of the functional podocin-nephrin complex: mutations in the *NPHS2* gene disrupt nephrin targeting to lipid raft microdomains. *Hum Mol Genet.* 2003;12:3397–3405
8. Ruf RG, Lichtenberger A, Karle SM, et al. Patients with mutations in NPHS2 (podocin) do not respond to standard steroid treatment of nephrotic syndrome. *J Am Soc Nephrol.* 2004;15: 722–732
9. Schultheiss M, Ruf RG, Mucha BE, et al. No evidence for genotype/phenotype correlation in *NPHS1* and NPHS2 mutations. *Pediatr Nephrol.* 2004;19:1340–1348
10. Weber S, Gribouval O, Esquivel EL, et al. NPHS2 mutation analysis shows genetic heterogeneity of steroid-resistant nephrotic syndrome and low post-transplant recurrence. *Kidney Int.* 2004;66:571–579
11. 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
12. Mucha B, Ozaltin F, Hinkes BG, et al. Mutations in the Wilms' tumor 1 gene cause isolated steroid resistant nephrotic syndrome and occur in exons 8 and 9. *Pediatr Res.* 2006;59: 325–331
13. Wagner N, Wagner KD, Xing Y, Scholz H, Schedl A. The major podocyte protein nephrin is transcriptionally activated by the Wilms' tumor suppressor *WT1*. *J Am Soc Nephrol.* 2004;15: 3044–3051
14. Guo G, Morrison DJ, Licht JD, Quaggin SE. *WT1* activates a glomerular-specific enhancer identified from the human nephrin gene. *J Am Soc Nephrol.* 2004;15:2851–2856
15. Haber DA, Buckler AJ, Glaser T, et al. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. *Cell.* 1990;61:1257–1269
16. Bruening W, Bardeesy N, Silverman BL, et al. Germline intronic and exonic mutations in the Wilms' tumour gene (*WT1*) affecting urogenital development. *Nat Genet.* 1992;1:144–148
17. Pelletier J, Bruening W, Li FP, Haber DA, Housman DE. *WT1* mutations contribute to abnormal genital system development and hereditary Wilms' tumour. *Nature.* 1991;353:431–434
18. Barbaux S, Niaudet P, Gubler MC, et al. Donor splice-site mutations in *WT1* are responsible for Frasier syndrome. *Nat Genet.* 1997;17:467–470
19. Zenker M, Aigner T, Wendler O, et al. Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet.* 2004;13: 2625–2632
20. Hasselbacher K, Wiggins RC, Matejas V, et al. Recessive missense mutations in *LAMB2* as a cause of isolated and non-Pierson type congenital nephrotic syndrome. *Kidney Int.* 2006; 70;1008–1012
21. Winn MP, Conlon PJ, Lynn KL, et al. A mutation in the TRPC6 cation channel causes focal segmental glomerulosclerosis. *Science.* 2005;308:1801–1804
22. Kaplan JM, Kim SH, North KN, et al. Mutations in *ACTN4*, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet.* 2000;24:251–256
23. Kim JM, Wu H, Green G, et al. CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science.* 2003;300:1298–1300
24. Karle S, Uetz B, Ronner V, et al. Novel mutations in *NPHS2* are

detected in familial as well as sporadic steroid-resistant nephrotic syndrome. *J Am Soc Nephrol.* 2002;13:388–393
25. Otto E, Hoefele J, Ruf R, et al. A gene mutated in nephronophthisis and retinitis pigmentosa encodes a novel protein, nephroretinin, conserved in evolution. *Am J Hum Genet.* 2002; 71:1161–1167
26. Till BJ, Burtner C, Comai L, Henikoff S. Mismatch cleavage by single-strand specific nucleases. *Nucleic Acids Res.* 2004;32: 2632–2641
27. Schumacher V, Scharer K, Wuhl E, et al. Spectrum of early onset nephrotic syndrome associated with *WT1* missense mutations. *Kidney Int.* 1998;53:1594–1600
28. Royer-Pokora B, Beier M, Henzler M, et al. Twenty-four new cases of *WT1* germline mutations and review of the literature: Genotype/phenotype correlations for Wilms tumor development. *Am J Med Genet A.* 2004;127:249–257
29. Holmberg C, Tryggvason K, Kestila M, Jalanko H. Congenital nephrotic syndrome. In: Avner E, Harmon W, Niaudet P, eds. *Pediatric Nephrology*. 5th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2004:503–516
30. McDonald R, Wiggelinkhuizen J, Kaschula RO. The nephrotic syndrome in very young infants. *Am J Dis Child.* 1971;122: 507–512
31. Huttunen NP. Congenital nephrotic syndrome of Finnish type: study of 75 patients. *Arch Dis Child.* 1976;51:344–348
32. Beltcheva O, Martin P, Lenkkeri U, Tryggvason K. Mutation spectrum in the nephrin gene (NPHS1) in congenital nephrotic syndrome. *Hum Mut.* 2001;17:368–373
33. Ohashi T, Uchida K, Uchida S, Sasaki S, Nihei H. Intracellular mislocalization of mutant podocin and correction by chemical chaperones. *Histochem Cell Biol.* 2003;119:257–264
34. Nishibori Y, Liu L, Hosoyamada M, et al. Disease-causing missense mutations in NPHS2 gene alter normal nephrin trafficking to the plasma membrane. *Kidney Int.* 2004;66:1755–1765

APPENDIX Clinical Data and Spectrum of Detected Sequence Variants in 89 Patients With NSFL

Variable	Family No.	Patient Reference	Gender	Ethnicity	Consanguinity	Age of Onset, y	First Clinical Presentation With	Extrarenal Manifestations	Biopsy	Therapy and Response	Age at Dialysis, y	Age at KT, y	ESRD After AO, y	No ESRD After AO, y	Status Last Seen	Sequence Variant	Consequence on Protein	Mutation Reference
CNS																		
NPHS1																		
	F212	3	M	Turkish	Y	0.0	NS	NN	CNF	NN	—	—	—	—	Deceased	[c.1135C>T] + [c.1135C>T]	[R379W] + [R379W]	1
	F228	3	M	German	N	0.0	Dystrophy	Mental retardation	DMS	ND	3.4	4.2	3.4	NA	KT, functional	[c.1738T>G] + [c.2479C>T]	[W580G] + [R827X]	1
	F294	3	M	Turkish	Y	0.0	NS, ED	—	ND	ND	3	4	3	NA	KT, functional	[IVS20 + 2T>A] + [IVS20 + 2T>A]	[Splice-site mutation] + [splice-site mutation]	1
	F435	3	M	Italian	NN	0.0	NS	NN	MCNS	NN	NN	NN	NN	NN	NN	[c.2617delTCAinsCC] + [c.2617delTCAinsCC] + NPHS2: [c.686G>A]	[F873fsX904] + [F873fsX904] + NPHS2: [R229Q]	2 + 3
	F439	3	M	German	Y	0.0	ED, premature	Growth retardation	ND	ND; NEC	2.9	3.8	2.9	NA	KT, functional	[c.1814delC] + [c.1814delC]	[A605fsX621] + [A605fsX621]	1
	F451	3	M	German	N	0.0	Elevated AFP, postnatal ED	Intracerebral haemorrhage	NN	NN	—	—	—	—	Deceased	[c.468C>G] + [IVS27 + 1G>T] + NPHS2: [c.686G>A]	[Y156X] + [Splice-site mutation] + NPHS2: [R229Q]	2 + 3
	F475 II-1	3	F	German	Y	0.0	Acute ED at birth	Failure to thrive	MCNS	SR	1.8	4.8	1.8	NA	KT, relapse/ failure	[c.2617delTCAinsCC] + [c.2617delTCAinsCC]	[F873fsX904] + [F873fsX904]	2
	F475 II-2	3	F	German	Y	0.0	Discrete ED at birth	Failure to thrive	ND	SR; CSA: NR	3.5	NA	3.5	NA	ESRD, no KT	[c.2617delTCAinsCC] + [c.2617delTCAinsCC]	[F873fsX904] + [F873fsX904]	2
	F571 II-1	3	F	Turkish	N	0.1	Acute ED, HT, hypocalcemia	Pyloric stenosis, bilateral hernia, connatal CMV infection	MCNS	SR; CSA: NR	<4	6	<4	NA	KT, functional	[c.1379G>A] + [c.1379G>A]	[R460Q] + [R460Q]	2
	F571 II-2		M	Turkish	N	0.0	ED, HT	—	ND	NN	5.5	NA	5.5	NA	ESRD, no KT	[c.1379G>A] + [c.1379G>A]	[R460Q] + [R460Q]	2
	F626	3	F	Turkish	Y	0.0	Congenital SP, SGA, PU, HU	Small for gestational age	MPCGP	SR	—	—	—	—	Deceased	[c.514 + 516delACC] + [c.514 + 516delACC]	[delT172] + [delT172]	4
	F806	3	F	Serbian	NN	0.2	ED	NN	MP	ND	7.8	9.1	7.6	NA	KT, functional	[c.3478C>T] + [c.3478C>T]	[R1160X] + [R1160X]	4
	F1017	3	M	German	N	0.0	NN	Failure to thrive	MS	ND	9.3	10.5	9.3	NA	KT, functional	[c.1258T>G] + [c.121 + 122delCT]	[F420V] + [L41fsX90] ("Fin major")	1 + 5
	F1093	3	F	Turkish	Y	0.1	ED, pallor	Prematurity, apnoe-bradykardia-syndrome, anaemia	CNF	ND	NA	NA	NA	3.2	No ESRD	[c.2014G>C] + [c.2014G>C]	[A672P] + [A672P]	1
	F1296 II-1	3	F	German	Y	0.1	ED	Connatal CMV infection, occipital aplasia cutis with bone defect	ND	ND	NA	NA	NA	1.5	No ESRD	[c.2617delTCAinsCC] + [c.2617delTCAinsCC] + NPHS2: [c.686G>A]	[F873fsX904] + [F873fsX904] + NPHS2: [R229Q]	2 + 3
	F1328 II-1	3	M	German	Y	0.0	ED	Mental retardation, hypertension	FSGS	SR; ACE-I	NA	NA	NA	25.2	No ESRD	[c.385C>T] + [c.1868G>T]	[L129F] + [C623F]	1 + 4
	F1328 II-3	3	F	German	Y	0.0	ED	—	NF	ND; ACE-I	NA	NA	NA	21.2	No ESRD	[c.385C>T] + [c.1868G>T]	[L129F] + [C623F]	1 + 4
	A229		M	Turkish	N	0.2	ED	—	ND	ND	NN	NN	NN	NN	?	[c.1001T>C] + [c.1001T>C]	[L334P] + [L334P]	13 + 13
	A650 II-1		M	Serbian	N	0.0	ED	Hydrocephalus, CNS hemorrhage, low birth weight	CNF	ND	—	—	—	—	Deceased	[c.614 + 621delCACCCCGGinsTT] + [c.614 + 621delCACCCCGGinsTT]	[T205+R207delinsI] + [T205+R207delinsI]	4

APPENDIX **Continued**

Variable	Family No.	Patient Reference	Gender	Ethnicity	Consanguinity	Age of Onset, y	First Clinical Presentation With	Extrarenal Manifestations	Biopsy	Therapy and Response	Age at Dialysis, y	Age at KT, y	ESRD After AO, y	No ESRD After AO, y	Status Last Seen	Sequence Variant	Consequence on Protein	Mutation Reference
	A797		F	German	N	0.0	Neonatal SP, PU	—	ND	NN	NA	NA	NA	0.4	No ESRD	[IVS27 + 1G>T] + [c.637C>T] + NPHS2: [c.686G>A]	[Splice-site mutation] + [Q213X] + NPHS2: [R229Q]	2 + 13 + 3
	A1028		M	German	N	0.1	PU, HT, ED	Thrombophilia	ND	ND	NA	NA	NA	0.7	No ESRD	[c.1248 + 1249insA] + [c.2607 + 2608insCC]	[T417fsX418] + [N870fsX905]	13 + 13
NPHS2																		
	F236 II-2	2	M	German	N	0.1	Acute ED, PU	NN	FSGS	SR	9.4	10.3	9.4	NA	KT, functional	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	F355 II-1	1, 2	M	German	N	0.0	PU, HT	NN	FSGS	SR; CSA: NR	9.8	9.9	9.8	NA	KT, functional	[c.460 + 461insT] + [c.413G>A]	[F155fsX167] + [R138Q]	3 + 6
	F355 II-2	1, 2	F	German	N	0.1	PU	Dual aortic arch	FSGS	ND	6.6	7.2	6.5	NA	KT, functional	[c.460 + 461insT] + [c.413G>A]	[F155fsX167] + [R138Q]	3 + 6
	F363		F	Italian	NN	0.0	NN	NN	ND	NN	NN	NN	NN	NN	NN	[c.417 + 418delG] + [c.417 + 418delG]	[L139fsX181] + [L139fsX181]	6
	F515 II-1	2	M	Czech	N	0.0	ED	Mental retardation	NCGP	SR	7.3	7.8	7.3	NA	KT, functional	[c.413G>A] + [c.417 + 418delG]	[R138Q] + [L139fsX181]	6 + 6
	F515 II-2	2	F	Czech	N	0.0	PU	—	CNF	SR	10	14.5	10	NA	KT, functional	[c.413G>A] + [c.417 + 418delG]	[R138Q] + [L139fsX181]	6 + 6
	F859	2	M	German	NN	0.0	PU, ED	NN	MCNS >> FSGS	SR; CSA: NR	7.2	NA	7.2	NA	ESRD, no KT	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	F876		M	German	N	0.0	ED	—	FSGS	ND	NA	NA	NA	4.1	No ESRD	[c.378G>T] + [c.378G>T]	[K126N] + [K126N]	7
	F935	2	M	German	N	0.0	ED	NN	minPGN	SR	NN	NN	NN	NN	NN	[c.460 + 461insT] + [c.506T>C]	[F155fsX167] + [L169P]	3 + 7
	F942 II-1	2	M	German	N	0.1	ED	Hypospadia	FSGS>> FGGS	ND	NN	NN	NN	NN	NN	[c.413G>A] + [c.503G>A]	[R138Q] + [R168H]	6 + 7, 8
	F942 II-2	2	M	German	N	0.1	ED	Hypospadia	ND	ND	NN	NN	NN	NN	NN	[c.413G>A] + [c.503G>A]	[R138Q] + [R168H]	6 + 7, 8
	F1028	2	M	German	N	0.0	Diagnosis intrauterine, acute ED	NN	MPGN	ND	NA	NA	NA	9.5	No ESRD	[c.353C>T] + [c.413G>A]	[P118L] + [R138Q]	7, 8 + 6
	F1221	2	M	German	N	0.0	ED, PU	Small stature	FSGS	ND	6.2	6.7	6.2	NA	KT, relapse/ failure	[c.378G>T] + [c.948delT]	[K126N] + [P316fsX347]	7 + 7
	F1233	2	F	German	N	0.0	PU, HU	Failure to thrive	ATGP	SR	NA	NA	NA	2.9	No ESRD	[c.413G>A] + [c.460 + 461insT]	[R138Q] + [F155fsX166]	6 + 3
	F1388		F	German	N	0.1	PU	Urinary tract infection	ND	ND	NA	NA	NA	0.8	No ESRD	[c.275G>A] + [c.413G>A]	[G92D] + [R138Q]	13 + 6
	A159		M	Czech	N	0.2	Micro-HU	—	FSGS	SR	NA	NA	NA	1.8	No ESRD	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	A237 II-1		F	Czech	Y	0.0	SP	—	CNF	SR; CP: NR	4.3	5.3	4.3	NA	KT, functional	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	A237 II-2		F	Czech	Y	0.0	PU, HU	—	ND	ND	NA	NA	NA	3.8	No ESRD	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	A248		M	German	N	0.2	PU	—	MCNS	SR	NN	NN	NN	NN	N	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	A356		M	German	N	0.0	Micro-HU, PU, HT	—	ND	SR	NN	NN	NN	NN	N	[c.412C>T] + [c.413G>A]	[R138X] + [R138Q]	6 + 6
	A674		F	German	N	0.2	ED, HT	Failure to thrive	MCNS >> FSGS	SR; CSA: CR	5.8	6.5	5.8	NA	KT, relapse/ failure	[c.413G>A] +[IVS4 + 1G>T]	[R138Q] + [Splice-site mutation]	6 + 3
	A887		F	German	N	0.0	ED	Craniofacial dysplasia	ND	NN	NA	NA	NA	0	No ESRD	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
WT1 and *LAMB2*																		
	F1012 II-1		F	Turkish	Y	0.2	ED, HT	—	FSGS	ND	—	—	—	—	Deceased	LAMB2: [c.737G>A] + [c.737G>A]	LAMB2: [R246Q] + [R246Q]	9

APPENDIX Continued

Variable	Family No.	Patient Reference	Gender	Ethnicity	Consanguinity	Age of Onset, y	First Clinical Presentation With	Extrarenal Manifestations	Biopsy	Therapy and Response	Age at Dialysis, y	Age at KT, y	ESRD After AO, y	No ESRD After AO, y	Status Last Seen	Sequence Variant	Consequence on Protein	Mutation Reference
	F1012 II-2		M	Turkish	Y	0.0	NS	—	NN	NN	NA	NA	NA	3	No ESRD	LAMB2: [c.737G>A] + [c.737G>A]	LAMB2: [R246Q] + [R246Q]	9
	F1234 II-2		M	German	N	0.0	NS	Eye anomalies, no mental retardation	NN	NN	NA	NA	NA	0.8	No ESRD	LAMB2: [c.961T>C] + [c.4177C>T]	LAMB2: [C321R] + [L1393F]	9
	A651		F	Serbian	N	0.0	ED	—	DMS	NN					Deceased	WT1: [c.1097G>A]	Ex8: [R366H]	10
Single sequence variant																		
	F920		F	German	N	0.0	ED	—	FSGS	SS: PR; CP: PR; CSA: PR	NA	NA	NA	15.4	No ESRD	NPHS2: [c.686G>A] + [?]	NPHS2: [R229Q] + [?]	3
	A816		M	Italian	N	0.0	ED	Deafness, cerebral palsy	PDT	NN	0.8	1.7	0	NA	KT, functional	NPHS1: [c.3478C>T] + [?]	NPHS1: [R1160X] + [?]	4
	A683		M	Turkish	Y	0.0	ED	Deafness, unilateral opticusatrophy, mental retardation, pulmonary valve stenosis, recurrence paresis	MS	NN	1.8	5	1.8	NA	KT, relapse/ failure	NPHS1: [c.3478C>T] + [?]	NPHS1: [R1160X] + [?]	4
	A917		F	Turkish	Y	0.1	ED, hypothyroid.	Strabism	FGGS	NN	NA	NA	NA	2.3	No ESRD	NPHS1: IVS4 + 2T>G + [?]	NPHS1: [splice-site mutation] + [?]	13
No mutation																		
	A163 II-1		M	Turkish	Y	0.1	ED	—	CNS	ND					Deceased	No mutation	No mutation	—
	F1373		M	German	N	0.3	ED	Kidney cysts, mental retardation	FSGS	SR; CP:NR; CSA:NR	17.4	NA	16.9	NA	ESRD, no KT	No mutation	No mutation	—
	A1018		M	Turkish	NN	0.0	ED, dystrophy	Failure to thrive	CNF	SR; CP:NR; CSA:NR	NA	NA	NA	0.1	No ESRD	No mutation	No mutation	—
Infantile nephrotic syndrome																		
	F1033		M	German	N	0.4	Enteritis, ED	—	ND	ND	NA	NA	NA	3.2	No ESRD	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	F398 II-3	1	F	German	N	0.6	ED	NN	MCNS >> FSGS	SR,CSA: NR	4.2	5	3.7	NA	KT, functional	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	F1030		F	Swiss	N	0.7	ED	NN	FSGS	SR, CP: NR	10	13.1 / 19.1	9.3	NA	KT, relapse/ failure	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	F1139		F	German	NN	0.7	PU, erythrocyturia, HT	NN	FSGS	SR, CP: NR	4	4.4	2.8	NA	KT, functional	[c.948del T] + [c.948del T]	[P316fsX347] + [P316fsX347]	7
	A126		M	German	NN	0.8	ED, HT	Short stature, vesico-urethral reflux	FSGS	SR	6.4	7.8	5.7	NA	KT, relapse/ failure	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	A900		F	German	N	0.8	UTI -> PU	Neurofibromatosis type I (suspected)	MCNS	SR, CP: NR					?	[c.G686A] + [c.1030 + 1031delT]	[R229Q] + [F343fsX347]	3 + 13
	F1336		F	Turkish	Y	0.9	ED, HT	CMV infection	MCNS	SR	NA	NA	NA	0.8	No ESRD	[c.G868A] + [c.G868A]	[V290M] + [V290M]	3
	F260 II-2	2	M	German	NN	1.0	ED, HT	—	NN	SR, CP: NR	8	8.5	7.0	NA	KT, functional	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6

APPENDIX **Continued**

Variable	Family No.	Patient Reference	Gender	Ethnicity	Consanguinity	Age of Onset, y	First Clinical Presentation With	Extrarenal Manifestations	Biopsy	Therapy and Response	Age at Dialysis, y	Age at KT, y	ESRD After AO, y	No ESRD After AO, y	Status Last Seen	Sequence Variant	Consequence on Protein	Mutation Reference
	F1023		M	German	NN	1.0	ED	—	FSGS	SR, CSA: NR	7	9.3	6.0	NA	KT, relapse/ failure	[c.413G>A] + [c.460 + 461insT]	[R138Q] + [F155fsX166]	6 + 3
	F1045	2	M	Turkish	NN	1.0	NN	Failure to thrive	FSGS	SR, CSA: NR	8.3	NA	9.0	NA	ESRD, no KT	[c.460 + 461insT] + [c.460 + 461insT]	[F155fsX166] + [F155fsX166]	3
	F1305		F	German	N	1.0	NN	—	FSGS	SR	4.2	6.3	3.0	NA	KT, functional	[c.413G>A] + [c.413G>A]	[R138Q] + [R138Q]	6
	A28 II-2		M	Turkish	Y	1.0	PU	—	MCNS	SR, CP:NR	NA	NA	NA	2.6	No ESRD	[c.460 + 461insT] + [c.460 + 461insT]	[F155fsX166] + [F155fsX166]	3
WT1 and *LAMB2*																		
	A794		F	Austria	N	1.0	ED, HT, hyperuricemia	—	DMS	ND	NA	NA	NA	0	No ESRD	WT1: [c.709G>C]	WT1: [E237Q]	11
	A1021		F	German	N	0.5	ED	—	ND	NN	NA	NA	NA	0	No ESRD	WT1: [c.1180C>G]	WT1: [R394P]	12
	F1234 II-1		F	German	N	0.3	ED	Eye anomalies, no mental retardation	MCNS	ND	NA	NA	NA	2.9	No ESRD	LAMB2: [c.961T>C] + [c.4177C>T]	LAMB2: [C321R] + [L1393F]	9
Single sequence variant																		
	F1313		F	German	N	0.3	ED, metabolic acidosis, seizure	Failure to thrive, mental retardation	MCNS	SS: PR; CSA: PR	NA	NA	NA	0.1	No ESRD	NPHS1: [c.791C>G] + [?]	NPHS1: [P264R] + [?]	13
	A276		M	Hungary	N	0.4	ED	—	FSGS	SR	NA	NA	NA	1.6	No ESRD	NPHS2: [c.686G>A] + [?]	NPHS2: [R229Q] + [?]	3
	A90		M	Turkish	N	0.8	ED	—	MPGN	SS	NA	NA	NA	3.3	No ESRD	NPHS2: [c.686G>A] + [?]	NPHS2: [R229Q] + [?]	3
No mutation																		
	A63		M	Turkish	Y	0.4	ED	Nephrocalcinosis, acidosis, facial dysmorphia	ND	ND	0.4	NA	0	NA	KT, functional	No mutation	No mutation	—
	A162		M	Turkish	N	0.4	ED, HT	NN	CNS	ND	NA	NA	NA	4	No ESRD	No mutation	No mutation	—
	F458		M	Italy	NN	1.0	Failure to thrive	Failure to thrive, mental retardation	FSGS	SR	NA	NA	NA	6.8	?	No mutation	No mutation	—
	F1224		M	Serbia	N	1.0	Acute: ED	NN	MHC	SS-SR, CP: NR, CSA: NR	NA	NA	NA	9.3	No ESRD	No mutation	No mutation	—
	F331		F	Turkish	Y	0.5	ED, ascites, HT	NN	FSGS	NN	0.7	NA	0.2	NA	Deceased	No mutation	No mutation	—
	F1396		M	German	Y	0.5	ED, HT	NN	FSGS	NN	NN	NN	NN	NN	?	No mutation	No mutation	—
	F1068		F	Turkish	Y	0.6	ED	NN	FSGS	SS: PR, CP: NN	NN	NN	NN	NN	?	No mutation	No mutation	—
	F287		F	Turkish	N	0.6	Urine analysis	NN	FSGS	SR,CP: NR, CSA: NR	NA	NA	NA	3.3	No ESRD	No mutation	No mutation	—
	F1368		M	German	N	0.7	ED, HT	NN	FSGS	SR, CT: NR	2.3	11.7	1.5	NA	KT, functional	No mutation	No mutation	—
	F1384		F	German	NN	0.8	ED	NN	ND	SS: PR	NA	NA	NA	0	No ESRD	No mutation	No mutation	—
	A1006		M	German	N	0.8	ED	NN	NN	NN	NA	NA	NA	0.3	No ESRD	No mutation	No mutation	—
	F247 II-2		F	German	N	1.0	NN	—	FSGS	SR	NA	NA	NA	0.4	Deceased	No mutation	No mutation	—
	F1074		F	Turkish	NN	1.0	ED	NN	FSGS	SR	NA	NA	NA	0	No ESRD	No mutation	No mutation	—
	A913		M	England	N	1.0	ED	blindness	DMS	NN	NN	12.6	11.6	NA	KT, functional	No mutation	No mutation	—
	A804 IV-6		M	England	N	1.0	ED, HT	NN	MGN	SR	NA	NA	NA	0.5	No ESRD	No mutation	No mutation	—

APPENDIX Continued

Variable	Family No.	Patient Reference	Gender	Ethnicity	Consanguinity	Age of Onset, y	First Clinical Presentation With	Extrarenal Manifestations	Biopsy	Therapy and Response	Age at Dialysis, y	Age at KT, y	ESRD After AO, y	No ESRD After AO, y	Status Last Seen	Sequence Variant	Consequence on Protein	Mutation Reference
	A1002		M	Turkish	N	1.0	ED	Foot malformation	FSGS	SR, CSA: NR	1.5	6	5	NA	KT, functional	No mutation	No mutation	—
	F445 II-1		M	Turkish	Y	1.0	ED, anuria	NN	NN	SS-SR	NA	NA	NA	17.1	No ESRD	No mutation	No mutation	—

References to patients include: (1) Karle S, Uetz B, Ronner V, et al. *J Am Soc Nephrol*. 2002;13:388–393; (2) Ruf RG, Lichtenberger A, Karle SM, et al. *J Am Soc Nephrol*. 2004;15:722–732; (3) Schultheiss M, Ruf RG, Mucha BE, et al. *Pediatr Nephrol*. 2004;19:1340–1348; (4) Mucha B, Ozaltin F, Hinkes BG, et al. *Pediatr Res*. 2006;59:325–331; and (5) Hasselbacher K, Wiggins RC, Matejas V, et al. *Kidney Int*. 2006;70;1008–1012. References to mutations include: (1) Schultheiss M, Ruf RG, Mucha BE, et al. *Pediatr Nephrol*. 2004;19:1340–1348; (2) Beltcheva O, Martin P, Lenkkeri U, Tryggvason K. *Hum Mut*. 2001;17:368–373; (3) Karle S, Uetz B, Ronner V, et al. *J Am Soc Nephrol*. 2002;13:388–393; (4) Lenkkeri U, Mannikko M, McCready P, et al. *Am J Hum Genet*. 1999;64:51–61; (5) Kestila M, Lenkkeri U, Mannikko M, et al. *Mol Cell*. 1998;1:575–582; (6) Boute N, Gribouval O, Roselli S, et al. *Nat Genet*. 2000;24:349–354; (7) Ruf RG, Lichtenberger A, Karle SM, et al. *J Am Soc Nephrol*. 2004;15:722–732; (8) Weber S, Gribouval O, Esquivel EL, et al. *Kidney Int*. 2004;66:571–579; (9) Hasselbacher K, Wiggins RC, Matejas V, et al. *Kidney Int*. 2006;70;1008–1012; (10) Royer-Pokora B, Beier M, Henzler M, et al. *Am J Med Genet A*. 2004;127:249–257; (11) Schumacher V, Scharer K, Wuhl E, et al. *Kidney Int*. 1998;53:1594–1600; (12) Breuning W, Bardeesy N, Silverman BL, et al. *Nat Genet*. 1992;1:144–148; (13) this study. ED indicates edema; HT, hypertension; HU, hematuria; NS, nephrotic syndrome; PU, proteinuria; SGA, small for gestational age; SP, sepsis; ACE-I, angiotensin-converting enzyme inhibitor; CR, complete response; CSA, Cyclosporine A; CP, Cyclophosphamide; NEC, nephrectomy; ND, not done; NN, not known; KT, kidney transplant; NR, no response; PR, partial response; SR, steroid resistant; SS, steroid sensitive; ATGP, Alport type glomerulopathy; CNF, Finnish type nephrotic syndrome; DMS, diffuse mesangial sclerosis; FGGS, focal global glomerulosclerosis; FSGS, focal segmental glomerulosclerosis; MCNS, minimal change nephrotic syndrome; MGN, membranous glomerulosclerosis; MHC, mesangial hypercellularity; MPGN, mesangio-proliferative glomerulosclerosis; MS, mesangial sclerosis; minPGN, minimal proliferative glomerulosclerosis; MPCGP, mesangial proliferative crescentic GN; MP, mesangial proliferation; NCGP, nonclassifiable glomerulopathy; NF, no findings; PDT, proliferation of distal tubulus; NN-ND, not known/not done; NN, not known; NA, not applicable; —, not calculated.