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ORIGINAL ARTICLE

Genetic abnormalities and prognosis in patients with congenital and infantile nephrotic syndrome

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Abstract
Background Congenital nephrotic syndrome (CNS) and infantile nephrotic syndrome (INS) are caused primarily by mutations in genes that encode structural and regulatory proteins of the glomerular filtration barrier. The aim of this study was to determine genotype–phenotype correlations and prognosis in patients with CNS and INS.
Methods *NPHS1*, *NPHS2*, *LAMB2* and the eighth and ninth exons of *WT1* were sequenced in 80 and 22 patients with CNS and INS, respectively. Genotype–phenotype correlations and survival were evaluated.
Results Causative mutations were identified in 64.7 % of patients, of which *NPHS1* mutations were the most common (37.4 %). The mutation detection rate was twofold higher in CNS patients than in INS patients (72.5 vs. 36.2 %). The most commonly mutated gene in CNS patients was *NPHS1* (46.3 %) versus *NPHS2* (13.6 %) and *WT1* (13.6 %) in INS patients. *NPHS2* mutations, female patients with *NPHS1* mutations, and *NPHS1* mutations affecting the transmembrane or intracellular domains of nephrin were associated with longer survival.
Conclusions Based on our present findings, the likelihood of identification of a genetic cause decreases with increasing age at diagnosis. The underlying genetic abnormality should be identified as early as possible, as this knowledge will facilitate clinicians in their prognostic prediction and enable patients to receive appropriate genetic counseling.

Keywords Congenital nephrotic syndrome · Infantile nephrotic syndrome · *NPHS1* · *NPHS2* · *WT1* · *LAMB2*

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Introduction

Nephrotic syndrome (NS) is a common chronic disease of childhood characterized by proteinuria, hypoalbuminemia, hyperlipidemia and edema [1]. Corticosteroids and other immunosuppressive medications are the mainstay of treatment; however, in NS patients with genetic defects that affect the structural and functional integrity of the glomerular filtration barrier (GFB) immunosuppressive treatments are generally known to be ineffective [2]. Such patients are at risk of side effects due to unnecessary immunosuppressive treatments. Consequently, the importance of genetic diagnosis in NS patients cannot be understated [3]. Congenital nephrotic syndrome (CNS) (age at onset of disease 0–3 months) and infantile nephrotic syndrome (INS) (age at onset of disease 4–12 months) are most commonly associated with mutations in genes that encode the structural and regulatory proteins of the GFB [4]. Mutations in several genes have been identified in CNS and INS patients, but most patients have mutations in one of four specific genes (*NPHS1*, *NPHS2*, *WT1* and *LAMB2*) [4, 5]. Hinkes et al. reported mutations in one of these four genes in 66 % of all CNS and INS cases in a predominantly

Central European patient population [6], but the relationship between genotype and prognosis in such patients has not been well documented. In this context, the aim of our study was to determine the frequency of mutations in these four genes (*NPHS1*, *NPHS2*, *WT1*, and *LAMB2*), genotype–phenotype correlations and prognosis in a large group of patients with CNS and INS.

Materials and methods

Patient group The study included 80 patients with CNS and 22 patients with INS. The ethnicity of the patients was Turkish (n=92), Syrian (n=4), Iranian (n =3), Serbian (n =2), and Bosnian and Herzegovinian (n =1). All patients were diagnosed with NS by a pediatric nephrologist and were referred to the Nephrogenetics Laboratory of the Department of Pediatric Nephrology, Hacettepe University, for genetic analysis. Clinical characteristics, treatment and survival information were obtained from physicians using standard clinical questionnaires. In the cases of patients lost to follow-up, their parents were interviewed by telephone to obtain the information on survival. The study protocol was approved by the Hacettepe University Ethics Committee (GO 13/46-28), and a completed informed consent form was obtained from each participant.

Genetic analysis Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. All exons and adjacent intronic boundaries of *NPHS1*, *NPHS2* and *LAMB2*, and exons 8 and 9 of *WT1* [7] were sequenced by the Sanger sequencing method using BigDye terminator v.3.1 sequencing kits and an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA). The primer pairs for the gene exons are available upon request. Raw data were analyzed using Sequencing Analysis Software and were compared to reference sequences in the Ensemble Database (http://www.ensembl.org/) for variations. DNA sequencing was also performed in the parents of the patients in whom a genetic alteration was identified in order to demonstrate segregation. All variations were searched for in the Human Gene Mutation Database Professional (http://www.hgmd.org/) (Access date: August 2014) and in earlier studies. In silico analyses using PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) and Mutation Taster (http://www.mutationtaster.org) were performed on novel variations to predict their pathogenicity.

Statistical analysis Patients were grouped according to mutated genes. Clinical findings are presented as the mean ± standard error of the mean, unless otherwise specified. Kaplan–Meier curves with the log-rank test were used to compare age at diagnosis and survival between groups. The level of statistical significance was set at $P<0.05$.

Results

The study included 102 patients [55 (54 %) male; 47 (46 %) female], of whom 80 patients had CNS and 22 had INS. Causative mutations were identified in one of the four genes studied in 66 (64.7 %) patients, as follows: bi-allelic mutations in *NPHS1* (n=38); bi-allelic mutations in *NPHS2* (n=16) and bi-allelic mutations in *LAMB2* (n =4); heterozygous mutations in *WT1* (n=8) (Tables 1, 2). The mutation detection rate was 72.5 % in the CNS patients versus 36.2 % in the INS patients (Table 1). The most commonly mutated gene in CNS patients was *NPHS1* (46.3 %) versus *NPHS2* (13.6 %) and *WT1* (13.6 %) in INS patients (Table 1).

Mean age at diagnosis of NS was lowest in the *NPHS1* group (1.1 months) followed by the *NPHS2* (2.6 months), *WT1* (3.9 months) and *LAMB2* (2.4 months) groups (Table 3). Kaplan-Meier analysis showed that patients in the *NPHS1* group had been diagnosed significantly earlier than those in the *NPHS2* and *WT1* groups (P=0.01 and P=0.006, respectively) (Fig. 1a). The consanguinity rate was high among all patients with gene mutations, but was lowest (50 %) in the *WT1* group (Table 3). Edema was the most frequent finding at presentation, regardless of gene mutation, but was most common (89 %) in the *NPHS1* group (Table 3). Among all groups, the median proteinuria grade was at least 3+. Microscopic hematuria was noted in 60 % of patients in the *NPHS1* group and in 60 % of those in the *NPHS2* group (Table 3). The mean serum creatinine level at initial presentation was >2 mg/dL in the *WT1* and *LAMB2* groups, and the mean serum albumin level was lowest (1.1 g/dL) in the *NPHS1* group (Table 3).

Extrarenal abnormalities were present in 41 % of the patients (33 % of those in the *NPHS1* group, 31 % of those in the *NPHS2* group, 50 % of those in the *WT1* group and 100 % of those in the *LAMB2* group). The most common extrarenal finding was congenital heart disease, manifesting in 13 % of patients in the *NPHS1* and *NPHS2* groups, respectively, and in 25 % of the patients in the *WT1* group. Of the latter, 25 % also had genital abnormalities (ambiguous genitalia, hypospadias and bifid scrotum) consistent with Denys–Drash syndrome and Frasier syndrome. One patient in the *WT1* group had stage-1 Wilms tumor and underwent unilateral nephroureterectomy. Patients with congenital heart defects had pulmonary stenosis (PS) (n=3), atrial septal defect (ASD) (n=3), and PS+ASD (n =3). In the *LAMB2* group, all patients had ocular abnormalities (microcoria and miosis) consistent with Pierson syndrome.

Patients received various kinds of medical treatment (Table 4), with 94 and 100 % of the patients in the *NPHS1* and *LAMB2* groups, respectively, treated with albumin, whereas the vast majority of patients in the *NPHS2* group were treated with immunosuppressive drugs (i.e. 60 % with corticosteroids, 30 % with cyclophosphamide, 30 % with

Table 1 Distribution of patients with congenital nephrotic syndrome and infantile nephrotic syndrome according to mutated genes or no diagnosed mutation

Syndrome	Mutated genes				No mutation	Total
	NPHS1	*NPHS2*	*WT1*	*LAMB2*		
CNS	37 (46.3%)	13 (16.2 %)	5 (6.2 %)	3 (3.8 %)	22 (27.5 %)	80 (100 %)
INS	1 (4.5 %)	3 (13.6 %)	3 (13.6 %)	1 (4.5 %)	14 (63.8 %)	22 (100 %)
Total	38 (37.4 %)	16 (15.6 %)	8 (7.8 %)	4 (3.9 %)	36 (35.3 %)	102 (100 %)

Data are presented as the number (of patients) with the percentage in parenthesis

CNS, Congenital nephrotic syndrome; INS, infantile nephrotic syndrome

cyclosporine A). Immunosuppressive medication was also commonly used in the *WT1* group (33 % with corticosteroids) (Table 4). All patients in the *WT1* group underwent peritoneal dialysis at an average 6.3 months of age. Compared to patients in the *WT1* group, fewer patients in the *NPHS1* and *NPHS2* groups required peritoneal dialysis (20 and 36 %, respectively) at a later age (17.2 and 64 months, respectively) (Table 4). Only one patient in the *LAMB2* group underwent peritoneal dialysis (at age 9 days). In all, seven patients in the *NPHS1* group and one patient in the *WT1* group underwent renal transplantation (Table 4).

The outcomes in 29 *NPHS1* patients, ten *NPHS2* patients, five *WT1* patients and three *LAMB2* patients were obtained. At the time this manuscript was prepared all ten patients in the *NPHS2* group were alive, whereas all patients in the *WT1* and *LAMB2* groups had died (mean age at death 6.1 and 4.0 months, respectively). In all, 52 % of the 29 patients in the *NPHS1* group died (mean age at death 5.6 months) (Table 4). The cause of death was identified in 17 of 23 patients as sepsis (15 patients), massive pulmonary embolism (1 patient) and sudden cardiac arrest at 4 days post-renal transplantation (1 patient). Three additional patients died at home due to unknown causes, and no information could be obtained on the cause of death of another three patients. Kaplan-Meier analysis showed that the 2-year survival rate was significantly higher in the *NPHS2* group than in all other groups (*NPHS2* vs. *NPHS1*, $P=0.01$; *NPHS2* vs. *WT1*, $P=0.0001$; *NPHS2* vs. *LAMB2*, $P=0.0004$) (Fig. 1b) and that survival in the *NPHS1* group was significantly longer than that in the *WT1* and *LAMB2* groups ($P=0.04$ and $P=0.02$, respectively). In the *NPHS1* group, females survived significantly longer than males ($P=0.01$) (Fig. 2a). Age at diagnosis was similar in males and females in the *NPSH1* group (Fig. 2b), and gender also had no effect on age at diagnosis or survival in the other groups (data not shown).

In the 66 patients, we identified 32 *NPHS1*, eight *NPHS2*, five *WT1* and four *LAMB2* mutations (Table 2), of which 14 mutations were novel [*NPHS1*($n=11$) and *LAMB2* ($n=3$)]. The most common *NPHS1* mutation was homozygous p.Arg1160Ter, which was identified in three patients from southeastern Turkey (of which 2 were siblings), one patient from Syria and one patient from Iran. The Fin-minor p.Arg1109Ter mutation was homozygous in two unrelated patients and heterozygous in one patient from the same town in western Turkey. The p.Gly394Val mutation was detected in two unrelated patients from the same city in northern Turkey. The affected domains of nephrin protein for the specific mutations are presented in Fig. 3. No association between the *NPHS1* mutation type (protein truncating or missense) and survival or age at diagnosis was found (data not shown), but the patients with mutations affecting transmembrane or intracellular domains of nephrin in ≥1 alleles had a significantly longer survival time than patients with mutations affecting the extracellular domain in both alleles (Fig. 4a; $P=0.04$). In addition, no association was found between the *NPHS1* mutation position and age at diagnosis (Fig. 4b).

The most common *NPHS2* mutation was p.Pro118Leu, which was identified in six unrelated patients from eastern and southeastern Turkey. The p.Leu156PhefsTer11 mutation was identified in four patients (2 of which were siblings) from Central Anatolia. There was no association between *NPHS2* mutation type (i.e. protein truncating or missense) and age at diagnosis (data not shown). Due to the small number of patients with *WT1* and *LAMB2* mutations, genotype–phenotype correlations could not be analyzed.

Discussion

The findings of our study show the genetic abnormalities and the association between genotype and clinical findings and prognosis in a large number of patients with CNS and INS. Mutations in *NPHS1*, *NPHS2*, *WT1* or *LAMB2* were identified in 64.7 % of the patients, which is in agreement with the findings of the earlier study by Hinkes et al. [6] which included patients who were predominantly Central European, in which 66 % of NS patients with disease onset during the first year of life had mutations in one of the same four genes. In our study the mutation detection rate was 72.5 % in patients with CNS versus 36.2 % in those with INS; both rates are slightly

Table 2 All of the gene mutations identified

Gene	Mutation	Zygosity[a]	Patients (*n*)	Reference
NPHS1	c.2664-4_2670del p.?	H	1	[27]
	c.2815+5G>A p.?	H	1	[28]
	c.2542A>C p.(Lys848Gln)/	Ch	1	None/[27]
	c.2549_2558del p.(Ala850GlufsTer52)		1	[27]
	c.1169A>G p.(Asp390Gly)	H	1	None
	c.866G>A p.(Trp289Ter)	H	1	[28]
	c.2206G>T p.(Val736Leu)	H	1	None
	c.3325C>T p.(Arg1109Ter)	H	2	[29]
	c.2404C>T p.(Arg802Trp)	H	1	[30]
	c.2800C>T p.(Gln934Ter)	H	1	none
	c.1099C>T p.(Arg367Cys); c.1135C>T p.(Arg379Trp)	Ch	1	[30]/[31]
	c.3478C>T p.(Arg1160Ter)	H	5	[30]
	c.3230A>G p.(Asn1077Ser)	H	1	[32]
	c.3233C>A p.(Ala1078Asp)	H	1	[32]
	c.2506+1G>T p.?	H	2	None
	c.1099C>T p.(Arg367Cys)	H	1	[30]
	c.2549_2558del p.(Ala850GlufsTer52)	H		[27]
	c.1181G>T p.(Gly394Val)	H	2	None
	c.614_621delinsTT p.(Thr205_Arg207delinsIle)	H	1	[30]
	c.1999C>A p.(Pro667Thr)/ c.2026C>A p.(Pro676Thr)	Ch	1	None/ none
	c.2014G>A p.(Ala672Thr); c.3233C>A p.(Ala1078Asp)	Ch	1	[8]/[32]
	c.3325C>T p.(Arg1109Ter); c.526_526+1del p.?	Ch	1	[29]/none
	c.1672C>T p.(Arg558Cys)	H	1	[31]
	c.1048 T>C p.(Ser350Pro)	H	1	[30]
	c.1223G>A p.(Arg408Gln)	H	1	[30]
	c.515_517del p.(Thr172del)	H	1	[30]
	c.2324G>T p.(Trp775Leu)	H	1	[8]
	c.2299C>T p.(Pro767Ser)	H	1	None
	c.526+2 T>G p.?	H	1	[6]
	c.609-2A>C p.?	H	1	[16]
	c.2549_2558del p.(Ala850GlufsTer52)/ c.3287del p.(Gly1096AlafsTer47)	Ch	1	[27]/none
	c.2881 T>C p.(Trp961Arg)	H	1	[8]
NPHS2	c.259G>T p.(Glu87Ter)	H	1	[33]
	c.353C>T p.(Pro118Leu)	H	6	[34]
	c.928G>A p.(Glu310Lys)	H	1	[8]
	c.467_468insT p.(Leu156PhefsTer11)	H	4	[35]
	c.503G>A p.(Arg168His)	H	1	[34]
	c.379G>T p.(Val127Phe)	H	1	[36]
	c.503G>A p.(Arg168His); c.809 T>A p.(Leu270Ter)	Ch	1	[34]/[37]
	c.413G>A (p.Arg138Gln)	H	1	[38]
WT1	c.1186G>C (p.Asp396His)	h	1	[39]
	c.1097G>A (p.Arg366His)	h	3	[40]
	c.1180C>T (p.Arg394Trp)	h	2	[40]
	IVS9+5G>T	h	1	[41]
	c.1186G>A (p.Asp396Asn)	h	1	[19]
LAMB2	c.1405+3A>T p.?	H	1	None
	c.4537C>T p.(Gln1513Ter)	H	1	None
	c.391del p.(Ile131LeufsTer20)	H	1	None
	c.459+2 T>C p.?	H	1	[42]

[a] H, Homozygote; h, heterozygote; Ch, compound heterozygote

lower than those previously reported [6, 8, 9]. We did not search for mutations in *PLCE1*, which have been reported to be the most frequent cause of diffuse mesangial sclerosis in patients with INS [10], nor did we search for other genes responsible for CNS and INS, such as those encoding endogenous synthesis of coenzyme Q10 [11, 12]. As such, it is possible that mutations in these genes, which could account for the CNS or INS in at least some of our patients, were overlooked. Other known genes which were not screened for, or as yet unknown genes, might also account for the relatively low mutation detection rate in the present study. As reported earlier [6, 9], the mutation detection rate among our patient cohort was 50 % lower in the INS patients than in the CNS patients, which strongly suggests that INS is genetically more heterogeneous than CNS and that some INS cases may be due to mutations in yet to be discovered genes. Non-genetic causes might also be an alternative explanation for the lower mutation detection rate in INS patients.

Most of the mutations in our study were identified in *NPHS1* (37.4 %), followed by *NPHS2* (15.6 %). Mutations in *NPHS1* were the most frequent cause of CNS in our patient cohort; this is in contrast with the findings of Hinkes et al. [6], who reported that mutations in *NPHS2* accounted for up to 51 % of all mutations in their Central European patient cohort with CNS. In that study, Turkish patients were analyzed separately, and no *NPHS2* mutations were identified in Turkish patients with CNS, whereas *NPHS1* mutations were the leading cause of CNS, as in the present study. In total, 32 *NPHS1* mutations were identified in the present study, of which 11 were novel. The Fin-major (p.Leu41AspfsTer50) mutation, which is the most common *NPHS1* mutation in Finland, was not identified in any of our patients, whereas we did identify the Fin-minor (p.Arg1109Ter) mutation in three patients (2 homozygous, 1 heterozygous) from the same town in western Turkey. In the present study, the most prevalent mutation in the *NPHS1* group was p.Arg1160Ter mutation (total of 5

Table 3 Demographic and renal findings at initial presentation among patients according to mutated genes

Demographic and renal findings	Mutated genes			
	NPHS1	*NPHS2*	*WT1*	*LAMB2*
Patients	38	16	8	4
Sex (male/female)	16/22 (42/58)	10/6 (63/37)	4/4 (50/50)	1/3 (25/75)
Consanguinity (%)	81	69	50	100
Age at diagnosis (months)	1.1±0.1	2.6±0.8	3.9±1.4	2.4±1.0
CNS/INS (%)	37/1 (97/3)	13/3 (81/19)	5/3 (63/37)	3/1 (75/25)
Edema (%)	89	56	75	75
Isolated proteinuria (%)	5	25	13	–
Median proteinuria level	3+	3+	3+	4+
Microscopic hematuria (%)	60	60	–	25
Serum creatinine (mg/dL)	0.35±0.7	0.41±0.1	2.14±0.4	2.04±0.9
Serum protein (g/dL)	2.87±0.1	4.47±0.2	3.46±0.4	2.73±0.2
Serum albumin (g/dL)	1.18±0.1	2.02±0.2	1.93±0.2	1.37±0.1

Data are presented as a number with/without the percentage in parenthesis, or as the mean ± standard error of the mean (SEM), where appropriate, unless indicated otherwise

CNS, Congenital nephrotic syndrome; INS, infantile nephrotic syndrome

patients: 3 from southeastern Turkey, 1 from Syria, 1 from Iran); this mutation has been reported to be associated with favorable prognosis in females [13]. Survival data were available only for the three Turkish patients (2 siblings and 1 unrelated). Among the siblings, the girl was 4.5 years old at the time this manuscript was prepared and did not require renal replacement therapy (RRT), whereas the boy died at the age of 3 months. The unrelated female patient died at age 8 months.

We found that our female patients with *NPHS1* mutations survived longer than their male counterparts, but that gender did not affect age at diagnosis. The observed gender-specific difference in survival in patients with an autosomal recessive inherited disease is interesting and leads us to believe that it is far beyond the known favorable prognosis of the p.Arg1160Ter mutation, which we identified in only five patients. For example, one female patient with a homozygous p.Ser350Pro mutation had a very benign disease course; at age 2 months she had acute onset edema, and laboratory analysis showed hypoalbuminemia (0.9 g/dL) and nephrotic range proteinuria (urinary protein/creatinine ratio 282 mg/mg creatinine). Corticosteroids were administered together with albumin infusions and angiotensin-converting-enzyme inhibitor, but remission could not be achieved with 4 weeks of corticosteroid treatment. Cyclosporine A treatment was then initiated, and partial remission was achieved in 3 months, together with resolving of the need for albumin infusion. At the time of writing this manuscript, the patient was 3 years old, had a normal glomerular filtration rate and did not require albumin infusion. An earlier study reported that female CNS patients with *NPHS1* mutations had slightly longer renal survival than males [8], and although we have no renal survival information, overall survival was longer in our female patients with *NPHS1* mutations than in their male counterparts—even though the treatment rate for both genders

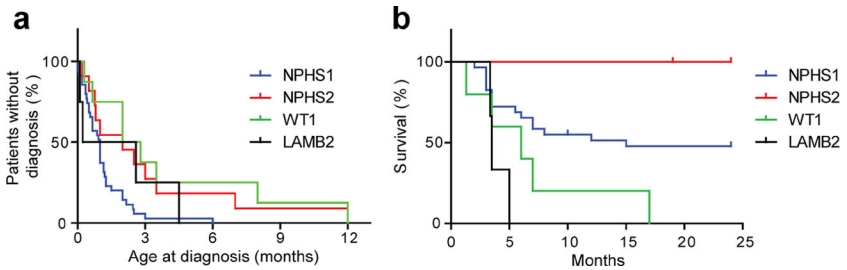

Fig. 1 **a** Age at diagnosis of nephrotic syndrome (NS), according to mutated genes. The *NPHS1* group had an earlier age at diagnosis than the *NPHS2* (log-rank test, $P=0.01$) and *WT1* groups (log-rank test, $P=0.006$). **a** Two-year survival curves for the NS patients according to mutated genes. The *NPHS2* group had a longer survival time than the *NPHS1* (log-rank test, $P=0.01$), *WT1* (log-rank test, $P=0.0001$) and *LAMB2* (log-rank test, $P=0.0004$) groups. The *NPHS1* group had a longer survival time than the *WT1* ($P=0.04$) and *LAMB2* groups ($P=0.02$)

Table 4 Treatments and prognoses of the patients according to mutated genes

Treatments and prognoses	Mutated genes			
	NPHS1	*NPHS2*	*WT1*	*LAMB2*
Albumin (%)	94	20	50	100
Corticosteroid (%)	9	60	33	–
Cyclophosphamide (%)	–	30	–	–
Cyclosporine A (%)	9	30	–	–
Angiotensin-converting-enzyme inhibitor (%)	44	20	67	100
Indomethacin (%)	9	–	–	–
Dialysis (%)	20	36	100	25
Age at dialysis onset (months)	17.2±2.0	64±9.6	6.3±2.5	0.3
Renal transplantation (%)	23	–	17	–
Age at renal transplantation (months)	32.4±4.6	–	60	–
Mortality (%)	52	–	100	100
Age at death (months)	5.6±0.6	–	6.1±1.5	4.0±0.4

Data are presented as a percentage or as the mean ± SEM, where appropriate

was similar [RRT for 30 % of male patients (mean age at the start of RRT: 16 months) and for 28 % of female patients (mean age at the start of RRT: 22.4 months)]. The albumin treatment rates were also similar for both female and male patients. Although unusual for an autosomal inherited disease, a gender-specific modifier effect has been reported for autosomal recessively inherited cystic fibrosis and neuronal ceroid lipofuscinosis [14, 15]. Gender differences in survival might be due to the effect of some modifier genes or hormonal factors. Further studies are required to reveal the underlying mechanisms of this gender effect on survival.

We did not find an association between *NPHS1* mutation type (i.e. protein truncating or missense), and age at diagnosis or survival, as previously reported by Machuca et al. [8]. These authors found that some patients with mutations affecting the intracellular domain of nephrin had a better clinical course [8]. In our study, CNS and INS patients with mutations affecting the transmembrane and intracellular domains of nephrin in ≥1 alleles had a longer survival time. Earlier functional studies have reported that mild missense mutations do not affect the targeting of nephrin to the plasma membrane, which causes partial preservation of protein function, whereas severe protein-truncating mutations cause intracellular trapping of the protein [8, 16]. Additional functional studies are needed to confirm our findings on the effect of mutation position on disease severity, especially in terms of the novel mutations described herein.

In the present study we identified eight *NPHS2* mutations that have previously been described elsewhere. Whereas the p.Arg138Gln mutation in *NPHS2* has been reported to occur in 50 % of all European CNS patients [8], this mutation was identified in only one of the patients in our study cohort. Also, in our Turkish patients, the most common *NPHS2* mutation was the p.Pro118Leu mutation, and all of these Turkish patients were from eastern and southeastern Turkey, suggesting that it is a founder mutation in those regions. The second most common mutation in the our patient cohort was the p.Leu156PhefsTer11 mutation, which was identified in four patients from central Anatolia. In a cohort of childhood and early adulthood onset steroid-resistant NS patients, Hinkes et al. [17] found that protein-truncating mutations in *NPHS2* were associated with an earlier diagnosis and worse prognosis, as compared to missense mutations. In our study we found no association between mutation type and age at diagnosis in the CNS and INS patients with *NPHS2* mutations (data not shown).

It has been reported that the long-term prognosis is poor in CNS and INS patients associated with *WT1* and *LAMB2* mutations. In these groups, end-stage renal disease (ESRD) may already be present at birth and may mask nephrotic symptoms [18, 19]. Genotype–phenotype correlation analysis in our patients with *WT1* and *LAMB2* mutations could not be performed due to the small number of patients; however, we did observe that these patients had a very high mean serum creatinine level at initial presentation and more commonly presented with chronic kidney disease, which is in agreement with previous reports [18, 19]. The mortality rates in both *WT1* and *LAMB2* patients were 100 % during the 2-year follow-up period. It has been reported that protein-truncating mutations in *LAMB2* are associated with an earlier age at NS diagnosis and ESRD as compared to missense mutations [18] and that missense

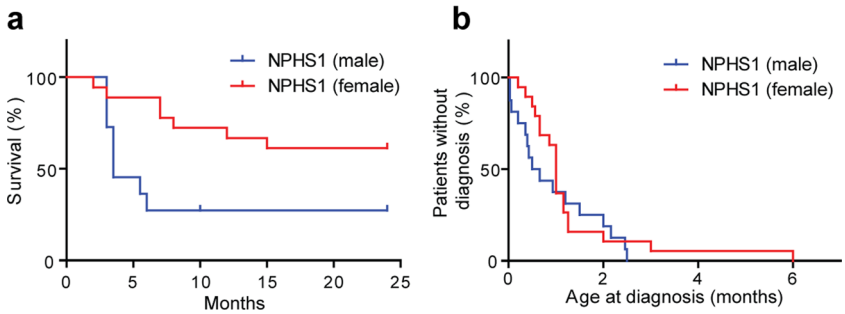

Fig. 2 Survival (**a**) and age at diagnosis (**b**) curves for the patients with *NPHS1* mutations according to gender. Female patients with *NPHS1* mutations survived longer than their male counterparts (log-rank test, $P=0.01$). Age at diagnosis was similar for both genders ($P>0.05$)

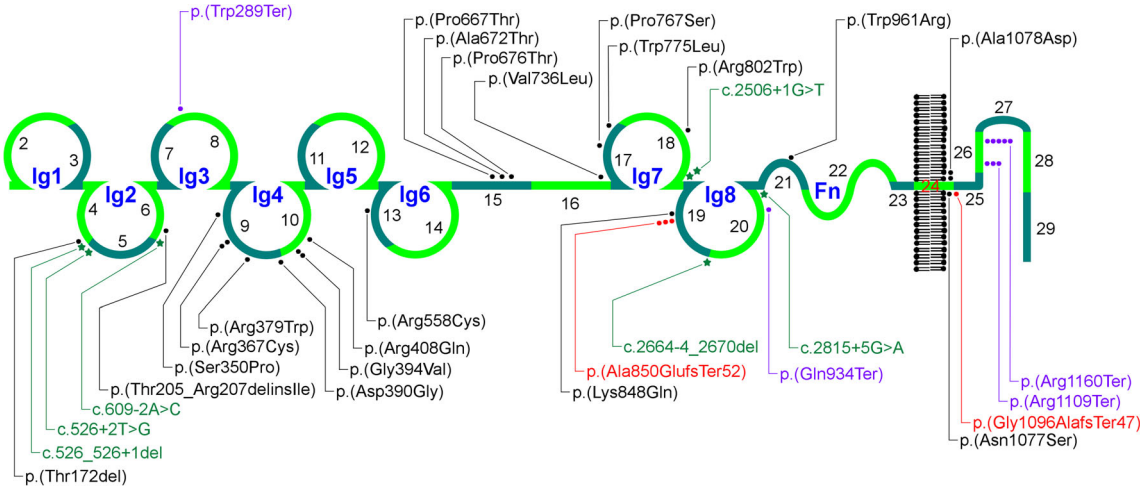

Fig. 3 Mutations identified in *NPHS1* and the corresponding affected parts of the protein. *Color coding* of mutations: *black* Mutations which affect 1–3 amino acids, *red* frameshift mutations, *purple* nonsense mutation, *green* splice-site mutations. Each dot represents a patient

mutations in *WT1* are associated with an earlier age at diagnosis and ESRD as compared to splice-site mutations [19]. We identified a total of two protein-truncating and two splice-site mutations in *LAMB2* and one splice-site and seven missense mutations in *WT1* in our patient cohort, which could explain the severe renal disease and poor outcome observed in the patients.

Turkey has a high prevalence of consanguineous marriage (20–25 %), which accounts for the high prevalence of rare recessive diseases [20, 21]. The consanguinity rate among the patients enrolled in our study was higher than that in the general Turkish population. Among the families studied, the consanguinity rate was highest in the recessively inherited groups [i.e. *NPHS1* (81 %), *NPHS2* (69 %), *LAMB2* (100 %)] and lowest in the *WT1* group (50 %), which is related to the presence of dominant or de novo mutations. In comparison, the consanguinity rate among the patients in our study without mutations in these four genes was 47 %, which suggests that NS might have been due to as yet unknown genetic abnormalities or non-genetic causes in these patients.

The 2-year survival in the *NPHS2* group was significantly longer than that in the *NPHS1*, *WT1* and *LAMB2* groups. We have no renal survival data. However, earlier reports suggest that renal survival in CNS patients with *NPHS2* mutations is better than that in patients with *NPHS1* mutations [8]. We believe that the longer survival time in the *NPHS2* group of the present study might be associated with a less severe clinical picture, as shown by higher serum albumin and/or lower serum creatinine levels at presentation in comparison to the other groups. Among all of patients, the 1- and 2-year survival rate was 56.5 and 52 %, respectively; these are lower than those which have been reported for West European CNS patients (1-year survival rate 79 %) [8]. Several factors might explain this discrepancy. First, comorbid conditions may be present; 41 % of our patients had extrarenal abnormalities (most commonly congenital heart defects [14 %]). Extrarenal findings associated with specific genetic abnormalities have also been identified in other studies in association with lower survival rates (i.e. 14 %; [8]). However, it is possible that the high consanguinity rate, which might also have caused coinheritance of two separate genetic defects, might have had an additional effect in our study patients. Second, the shorter survival in our Turkish patients

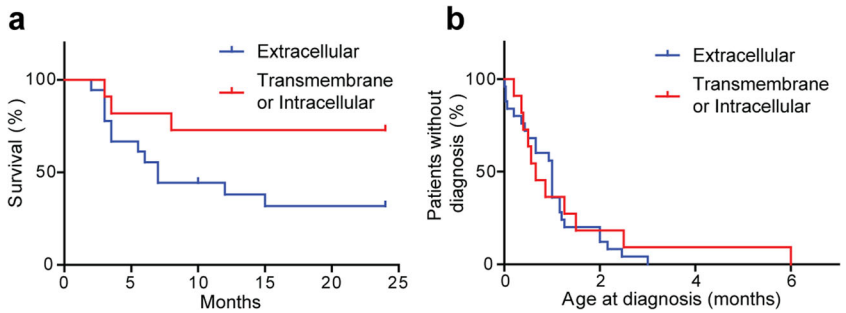

Fig. 4 Survival (**a**) and age at diagnosis (**b**) curves for the patients with *NPHS1* mutations, according to mutation position. Patients with mutations affecting the transmembrane or intracellular domains of the nephrin protein in ≥1 alleles survived longer than patients with homozygous or compound heterozygous mutations affecting the extracellular domain (log-rank test, $P=0.04$). Age at diagnosis was similar in both groups ($P>0.05$)

might be due to the treatment modalities. Although 94 % of the *NPHS1* patients and 100 % of the *LAMB2* patients were treated with albumin; none underwent nephrectomy, which is widely performed in countries where CNS is prevalent [22, 23].

Interestingly, 13 % of both the *NPHS1* and *NPHS2* patients and 25 % of the *WT1* patients had congenital heart defects, including PS, ASD and both. Congenital heart defects have been reported in patients with *NPHS1* and *NPHS2* mutations [8, 24]. In addition, earlier studies have demonstrated that the *NPHS1* and *WT1* genes play a role in cardiac embryological development [25, 26]. Thus, it is possible that mutations in these genes can cause susceptibility to congenital heart defects—but this requires further research.

In conclusion, the findings from our study indicate that the likelihood of an underlying genetic abnormality is inversely related to age in NS patients. Although the mutation detection rate was more or less similar in all of the ethnic groups, the underlying genetic abnormalities responsible for NS during the first year of life may be different. Consequently, it is important that the genotype should be determined in each race in order to provide the best possible follow-up and outcome. Female patients with *NPHS1* mutations and with *NPHS1* mutations affecting the transmembrane or intracellular domains of nephrin were associated with longer survival. In nephrotic patients aged <1 year, clinicians should make every effort to identify the underlying genetic abnormality, which can facilitate the prediction of prognosis, rational treatment and appropriate genetic counseling.

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