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ORIGINAL ARTICLE

Novel mutations in steroid-resistant nephrotic syndrome diagnosed in Tunisian children

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Abstract Steroid-resistant nephrotic syndrome (NS) remains one of the most intractable causes of end-stage renal disease in the first two decades of life. Several genes have been involved including *NPHS1*, *NPHS2*, *WT1*, *PLCE1*, and *LAMB2*. Our aim was to identify causative mutations in these genes, in 24 children belonging to 13 families with NS manifesting with various ages of onset. We performed haplotype analysis and direct exon sequencing of *NPHS1*, *NPHS2*, *PLCE1*, *LAMB2,* and the relevant exons 8 and 9 of *WT1.* Ten different pathogenic mutations were detected in seven families concerning four genes (*NPHS1* (3/7), *LAMB2* (2/7), *NPHS2* (1/7), and *WT1* (1/7)). Five of the detected mutations were novel; IVS9+2 T>C and p.D616G in *NPHS1*; p.E371fsX16 in *NPHS2*, and p. E705X and p.D1151fsX23 in *LAMB2.* Nine of 24 patients failed to be categorized by mutational analysis. Our study extends the spectrum of abnormalities underlying NS, by reporting novel mutations in the *NPHS1* and *NPHS2* genes and the first cases of *LAMB2* mutations in Tunisia. Congenital and infantile NS can be explained by mutations in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* genes. The identification of additional genes mutated in NS can be anticipated.

Keywords *LAMB2* · Mutations · Nephrotic syndrome · *NPHS1* · *NPHS2*

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Introduction

Nephrotic syndrome (NS) is a clinically heterogeneous disease characterized by different histological variants [1–3] and genetic determinants [4, 5]. Clinically, it is split into steroid-sensitive and steroid-resistant forms [6]. In children, steroid-resistant NS (SRNS) may be congenital NS (CNS), manifesting in utero or during the first 3 months of life or infantile, uncovered in the first year of life. It may also occur later in life. Several genes are involved in genetic forms of NS occurring in children. They encode proteins highly expressed in the glomerular filtration barrier [6]. The majority of genes associated with NS [7] code for structural elements of the slit diaphragm (SD) (*NPHS1*, *NPHS2*, *CD2AP*) and podocyte (*PLCE1*). Other encoded proteins expressed in the glomerular basement membrane (GBM; *LAMB2*) [8] or transcription factors necessary for normal podocyte function and glomerular development (*WT1*). Mutations in *NPHS1*, *NPHS2*, and *PLCE1* genes were found in most of the severe isolated cases of congenital and early onset NS whereas *LAMB2* and *WT1* were implicated in syndromic forms of NS [6].

Nephrin, the product of the gene *NPHS1* mutated in CNS of the Finnish type [9], was identified as the major

component of the SD and plays a significant role in signaling processes that are important for podocyte function [7]. The second essential component of the SD and a close interactor of nephrin is podocin encoded by *NPHS2* [5]. *NPHS2* mutations are a common cause of childhood SRNS, but they are also important in the development of CNS [10, 11]. Phospholipase C epsilon 1 (PLCε1) [12], encoded by the *PLCE1* gene, also appeared to be a crucial podocyte protein. *PLCE1* mutations [13] have been described in early onset NS. CD2-associated protein (CD2AP) is an adapter molecule originally identified as a ligand for the T cell-adhesion protein CD2. In spite of the clear association of CD2AP defects with glomerular disease in animal models [14], little is known about the human phenotype associated with *CD2AP* mutations [15]. Mutations in *WT1* and *LAMB2* are mostly observed in syndromic NS. *WT1* (Wilms tumor 1) mutations are associated with the Denys–Drash (DDS) [16] and Frasier syndromes (FS) [17], whereas Pierson syndrome [18] is caused by mutations in the *LAMB2* gene that codes for laminin β2, which is widely expressed in the GBM and is crucial for podocyte foot process architecture and stability, and also in other organs such as the eye. Phenotypes of variable severity have been described [19].

The aim of this study was to identify causative mutations in a Tunisian cohort of 24 children with sporadic or familial NS.

Patients and methods

Patients

Between 2002 and 2009, 24 children belonging to 13 families (8 of whom were consanguineous) and suffering from either sporadic or familial NS were included in the study (Table 1). They were admitted by pediatric nephrologists from specialized centers in Tunisia for congenital or SRNS. Diagnosis of NS and response to steroid treatment were classified according to published criteria [20]. In this analysis we collected the following data: age of NS onset, clinical symptoms (edema, blood pressure), urine status (proteinuria, hematuria), extrarenal symptoms (eye abnormalities, urogenital abnormalities), renal function, response to steroid therapy and to alternative drug treatment (alkylating agents, cyclosporine), and report of the renal biopsy when performed. Renal biopsy specimen, were analyzed by one reference pathologist. When children reached end stage renal disease (ESRD), they were offered dialysis pending a renal transplantation. Patient outcome was classified according to the patient's renal function status (Table 1).

Renal biopsy

Seventeen out of 24 children underwent percutaneous renal biopsy within 3 months of the diagnosis of NS. The procedure was performed under cœlioscopy or ultrasound guidance with the consent of patients' parents. The specimens were processed for light microscopy.

Molecular genetic study

After obtaining parental consent, genomic DNA was isolated from peripheral blood leucocytes, as described previously [21].

Four polymorphic microsatellite markers flanking for several genes of NS were tested (Table 2). The age of onset, the clinical signs, renal and extra-renal signs, and the report of the renal biopsy were the elements of the basis for the choice of genes to be tested. For patients with congenital NS, linkage analysis was performed for *NPHS1* and *NPHS2*, as the first choice, and for *LAMB2* in patients with ocular anomalies. If no mutation was detected, linkage analysis for *PLCE1* and *CD2AP* was performed. However, for patients with infantile and later onset NS, linkage analysis for *NPHS2* and *PLCE1* was the first step. If no mutation was detected, linkage analysis for *NPHS1* and *CD2AP* was performed. Genotyping was performed after PCR amplification and electrophoresis. Cyrillic 2.1 (Cherwell Scientific, Oxford, UK) was used to construct pedigrees and to perform haplotype analysis. In families potentially linked to one gene, direct sequencing of the coding exons and the adjacent intronic junctions was performed. PCR products were treated with Exo-SAP IT (GE Healthcare, Buckinghamshire, UK), and both strands were sequenced using the dideoxy chain termination method on a 3130 XL DNA sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed using Sequencher 3.1 software (Genecodes, Ann Arbor, MI, USA). Amino acid conservation and the potentially damaging effect of missense mutations were assessed using PolyPhen-1 (polymorphism phenotyping) software [22]. PolyPhen designates variants as either probably or possibly damaging or benign on the basis of sequence annotation, sequence alignment, and structural parameters [23]. Donor and acceptor sites for splicing were predicted by NetGene2 [24].

Results

A total of 13 families, with 24 affected individuals (10 girls, 14 boys) and 14 healthy siblings were included in the present study. Twelve had CNS (5 of them born prematurely). Two had infantile NS. Ten had childhood-onset NS.

Table 1 Pathological and mutational analysis relationship

	Patient	Sex	Age at diagnosis	Consanguinity	Nucleotide alteration(s)	Effect on protein	Mutation status	Renal biopsy	Current status/age of death or LF	References
					NPHS1					
FI	1	Male	Neonatal	Yes	c.[1847 A>G]+[1847 A>G]	p.D616G	hom	MH	Died (2 months)	PR
FII	1	Male	2 months	Yes	c.[1170+2 T>C]+[1170+2 T>C]	Splice site	hom	ND	Died (3 month)	PR
	2	Male	2 months	Yes	c.[1170+2 T>C]+[1170+2 T>C]	Splice site	hom	NTL	Died (3 months)	PR
FIII	1	Male	3 months	Yes	c.[614_621del8insTT]+[1379 G>A]	p.T205_R207delinsI+ p.R460Q	c het	ND	Died (4 months)	[4, 25, 26]
					LAMB2					
FIV	1	Male	40 days	No	c.[3450_3451 ins A]+[536 C>T]	p.D1151fsX23+ p.S179F	c het	DMS	Died (20 months)	PR [27]
	2	Male	30 days	No	c.[3450_3451 ins A]+[536 C>T]	p.D1151fsX23+ p.S179F	c het	DMS	Died (3 months)	PR [27]
FV	1	Male	At birth	Yes	c.[2113 G>T]+[2113 G>T]	p.E705X	hom	DMS	Died (27 days)	PR
	2	Male	At birth	Yes	c.[2113 G>T]+[2113 G>T]	p.E705X	hom	ND	Died (0 days)	PR
	3	Female	At birth	Yes	c.[2113 G>T]+[2113 G>T]	p.E705X	hom	ND	Died (2 days)	PR
	4	Male	At birth	Yes	c.[2113 G>T]+[2113 G>T]	p.E705X	hom	ND	Died (13 days)	PR
	5	Male	At birth	Yes	c.[2113 G>T]+[2113 G>T]	p.E705X	hom	ND	Died (10 days)	PR
	6	Female	At birth	Yes	c.[2113 G>T]+[2113 G>T]	p.E705X	hom	DMS	Died (30 days)	PR
					NPHS2					
FVI	1	Female	10 years	No	c.[538 G>A]+ [1113_1119del GCCACTA]	p.V180M+ p.E371fsX16	c het	FSGS	Died (10 years)	[5] PR
	2	Female	15 years	No	c.[538 G>A]+ [1113_1119del GCCACTA]	p.V180M+ p.E371fsX16	c het	FSGS	On dialysis	[5] PR
					WT1					
FVII	1	Female	6 months	No	c.[1097 G>A]	p.R366H	het	DMS	CT (LF:12 months)	[28]
					No mutation					
FVIII	1	Male	8 months	No		–	–	FSGS	Died (12 months)	
FVIIII	1	Female	10 years	No		–	–	FSGS	On dialysis	
FX	1	Male	5 years	Yes		–	–	FSGS	On dialysis	
FXI	1	Male	3 years	Yes		–	–	FSGS	On dialysis	
FXII	1	Male	10 years	Yes		–	–	FSGS	On dialysis	
FXIII	1	Female	8 years	Yes		–	–	FSGS	On dialysis	
	2	Female	15 years	Yes		–	–	FSGS	On dialysis	
	3	Male	17 years	Yes		–	–	ND	CT (LF:18 years)	
	4	Male	19 years	Yes		–	–	FSGS	Transplanted (23 years)	

FSGS focal segmental glomerulosclerosis, *DMS* diffuse mesangial sclerosis, *MH* mesangial hypercellularity, *CT* conservative treatment, *LF* last follow-up, *ND* not done, *c het* compound heterozygous mutations, *NTL* nonspecific tubular lesions, *PR* present report

Table 2 Polymorphic microsatellite markers

Gene	Microsatellite markers
NPHS1	D19S425, D19S425, D19S610, D19S608
NPHS2	D1S212, D1S3760, D1S215, D1S2751
PLCE1	D10S185, D10S1680, D10S574, D10S1726
LAMB2	D3S 3640, D3S3560, D3S3629, D3S1573
CD2AP	D6S452, D6S1689, D6S16, D6S438

The median age of the first symptoms was 17 days, 7.5 months, and 10.5 years respectively. FSGS lesions were found in 10 out of 17 children, diffuse mesangial sclerosis (DMS) lesions were found in 5 out of 17 cases and specific CNS lesions of the Finnish type were observed in 1 out of 17. Nonspecific renal lesions were observed in 1 out of 17.

Ten different pathogenic mutations (5 missense, 3 nonsense, 1 splice site, and 1 deletion insertion mutation) were detected in different NS genes (Figs. 1, 2). Five of them were novel mutations and were absent in 100 healthy Tunisian control individuals.

In terms of families, *NPHS1*, *LAMB2*, *NPHS2*, and *WT* mutations were identified in 3/13, 2/13, 1/13, and 1/13 respectively. The median age of onset of disease in children with mutations was 19.1 months. No mutation was detected in the *PLCE1* and *CD2AP* genes.

NPHS1 mutations

Four mutations were identified in three unrelated consanguineous families, two of which were novel. One was a missense mutation, c.1847A>G in exon 14, predicting the amino-acid exchange p.D616G with a PolyPhen score of 1.99. The other was a splice site mutation c.1170+2 T>C in the intronic splicing region of exon 9 (IVS9 +2 T>C). These two mutations were detected in patients FI-1, FII-1, and FII-2 respectively. The in-frame deletion insertion c.614_621del8insTT (p.T205_R207delinsI) in exon 6 [4] was detected in patient FIII-1, although they originated from a consanguineous family. It appeared to be a compound heterozygote for the c.614_621del8insTT mutation and the p.R460Q missense mutation, which had previously been reported in European patients [25]. This patient was also included in the study by Machuca et al. [26]. The 4 patients were admitted to the neonatal unit, at a median age of 35 days, for generalized edema and severe NS. Renal biopsy was performed only in 2 patients. Proximal tubule dilatation and mesangial hypercellularity of glomeruli were shown in patient FI-1. However, lesions of renal parenchyma were moderate and not specific in patient FII-2. The treatment was symptomatic without corticotherapy. End-stage renal failure (ESRF)

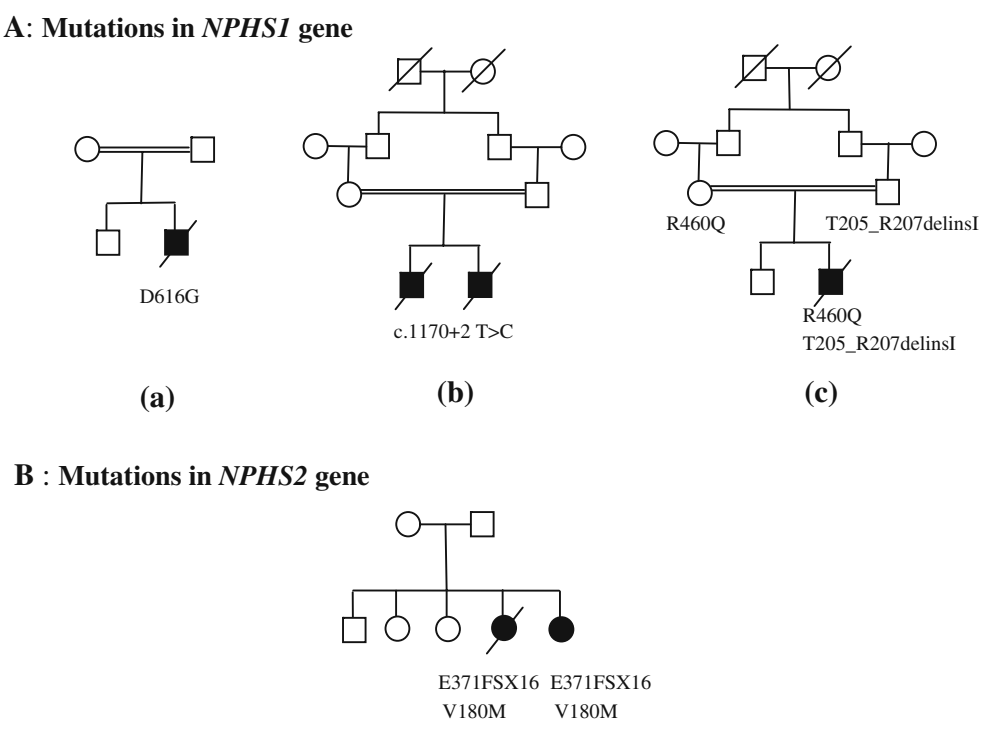

Fig. 1 Mutations detected in *NPHS1* and *NPHS2* genes. **A** Mutations in the *NPHS1* gene. **a** Mutation in exon 14 in family FI. **b** Mutation in the intronic splicing region of exon 9 in family FII. **c** Mutation in family FIII: missense mutation in exon 6, a deletion-insertion mutation in exon 11. **B** Mutations in the *NPHS2* gene in family FVI: missense mutation in exon 5, truncation mutation in exon 8

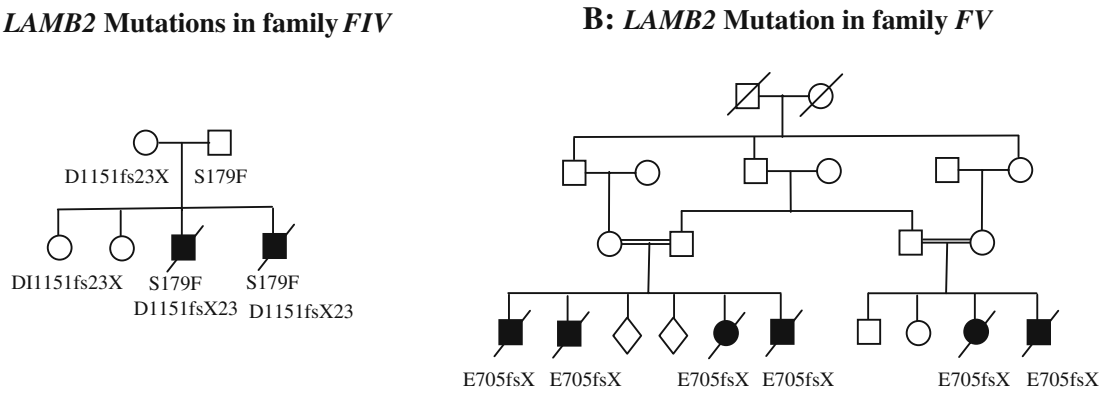

Fig. 2 *LAMB2* mutations detected in families FIV and FV. **A** Mutations in family FIV: missense mutation in exon 5, truncation mutation in exons 23–24. **B** Mutation in exon 16 in family FV

was reached at a median age of 40 days. All patients died from their disease at the median age of 2 months after birth.

NPHS2 mutations

Two affected sisters of family FVI were compound heterozygous for two *NPHS2* mutations. A novel deletion, c.1113_1119delGCCACTA, was detected in exon 8, resulting in a premature termination codon (p.E371fsX16) in the C-terminal part of the protein. The second mutation, in exon 5, resulting in the p.V180M change, had been previously described [5]. Both affected patients had a late onset steroid-resistant NS (a median age of 10 years) and reached ESRF at the median age of 11.5 years. Renal biopsy showed mesangial proliferation with FSGS. FVI-1 died at the age of 10 years. FVI-2 is still on dialysis, waiting for renal transplantation.

LAMB2 mutations

Three sequence variations were identified in the coding sequence of the *LAMB2* gene, two of which were novel. The first one was a nonsense mutation, c.2113 G>T, affecting exon 16 and predicting a protein truncation p.E705X. It was detected in the homozygous state in affected children of family FV. The two other mutations were compound heterozygous sequence variations, detected in the affected members of family FIV (FIV-1 and IV-2). One variant was a maternally inherited insertion in exon 24 (c.3450_3451insA), causing a protein truncation, p.D1151fsX23. The other mutation was a heterozygous missense mutation in exon 5 (c.536 C>T) leading to the substitution of phenylalanine for serine at position 179 (p.S179F). Being predicted to be possibly damaging by PolyPhen (score 1.56) has already been reported in a patient with Pierson syndrome [27]. Very severe CNS was identified in FIV and FV-affected individuals. Family FIV was a nonconsanguineous family, with two affected children. They developed proteinuria before the age of 3 months (40 and 30 days respectively) and ESRF within the first years of life (20 and 3 months respectively). Kidney biopsy showed a severe glomerular nephropathy, as seen in Pierson syndrome with the association of podocytosis, capillary retraction, and anomalies of capillary membranes in the absence of mesangial expansion (Fig. 3). FIV-1 was noted to have megalocornea with dysgenetic angle. For patient FIV-2, ophthalmic data were incomplete. The patients died at the age of 20 months and 3 months with nosocomial infection and with pulmonary hemorrhage respectively.

In family FV, the parents were first cousins. All 4 affected children (FV1–4) and 2 cousins (FV5–6) suffered from prematurity, very severe CNS, with exophthalmia,

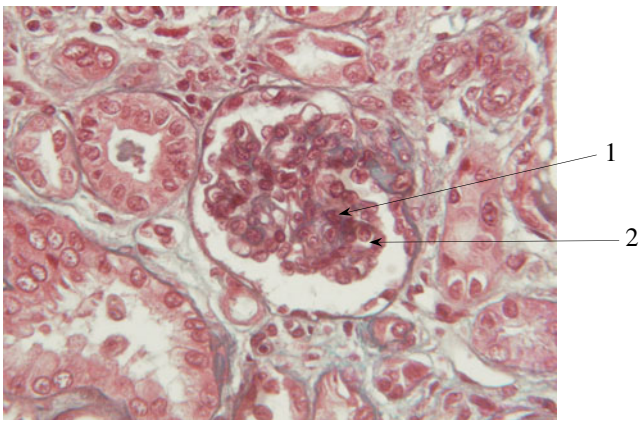

Fig. 3 Kidney biopsy of patient FIV-1, with *LAMB2* mutations (p.S179F and p.D1151fsX23). Glomerular nephropathy, with podocytosis (*1*), capillary retraction, and anomalies of capillary membranes (*2*) in the absence of mesangial expansion

facial dysmorphy, asymmetric implantation, and low-set ears. Kidney biopsy was performed only in patient FV6 and showed DMS. All patients died in the first days of life, at a medium age of 13.8 days after birth.

WT1 mutation

We detected a de novo mutation, p.R366H in exon 8, in patient FVII-1 belonging to a nonconsanguineous family. This dominant heterozygous mutation has been previously reported [28] and is known to be associated with DDS. First symptoms occurred at the age of 6 months and DMS was observed on early biopsy specimens. The child was a girl, without urogenital malformation. No development of Wilms tumor or any other extrarenal symptoms was noted. At the last follow-up examination (at 1 year old), no progressive renal degradation was observed.

Subjects with unidentified mutations

Nine of the 24 patients (37.5%) belonging to families FVIII to FXIII were genotyped for the *NPHS1*, *NPHS2*, *PLCE1*, *LAMB2*, and *CD2AP* genes.

Families FVIII, FVIII, and FX were potentially linked to the *NPHS1*, *NPHS2*, *PLCE1*, and *CD2AP* genes and were sequenced for all these genes. No linkage for any of the studied genes was found in FXI and FXII. The family FXIII was linked only for *NPHS1* and *CD2AP* and so these genes were sequenced for this family.

No pathogenic mutation was detected after the sequencing of the linked genes. Whole genome-wide linkage analysis is considered for the consanguineous family FXIII, with 4 affected children.

The median age of onset of disease in these children was 9 years (range 8 months to 17 years). Only one patient (FVIII-1) presented infantile NS and died at the age of 12 months. The others presented childhood NS and reached ESRF at a median age of 9.1 years. Renal biopsy showed mesangial proliferation with FSGS in all patients. Currently, 1 patient is still on conservative treatment (last follow-up at 18 years), 6 out of 8 underwent dialysis (median age of 11 years), and 1 patient was transplanted with no recurrence of the disease at the last follow-up (at the age of 24 years).

Discussion

In recent years, several podocyte genes have been implicated in different forms of NS progressing to ESRF. In this study, we present results of mutation screening of *NPHS1*, *NPHS2*, *WT1*, *LAMB2*, and *CD2AP* genes performed in 24 Tunisian children from 13 families affected with NS. Fourteen patients had early onset NS, with clinical signs starting at a mean age of 1 month (range 0 to 5 months).

Mutations were found in 53.8% of our limited cohort, and, interestingly, in all families with CNS. Classically, this range of patients carries mutations in the *NPHS1* or *NPHS2* genes. In our study, *NPHS1* mutations were the most frequently detected form (23%); however, *NPHS2* mutations were less frequent than hypothesized (7.6%). Interestingly, *LAMB2* mutations represented the second most frequent cause of CNS detected in our families (15.3%).

We observed 4 *NPHS1* mutations, of which 2 were novel, a splice site mutation (IVS9 +2 T>C), and a missense mutation (p.D616G) altering the 6th Ig-like domain. The in-frame mutation p.T205_R207delinsI, and a missense mutation p.R460Q, were previously reported in European patients with CNS [4, 25]. Interestingly, these two mutations were found in patient FIII-1, who belonged to a highly inbred family. In spite of the absence of homozygosity in the patient haplotypes at the *NPHS1* locus, *NPHS1* was sequenced. It has been reported [29] that the occurrence of allelic heterogeneity within a consanguineous community may account for the absence of homozygosity in flanking markers and consequently the failure to detect linkage. This could lead to the false conclusion that the disorder studied is not mapped to a known genetic locus. Similar cases of multiple rare mutations were reported in several inbred communities with some recessive diseases [30–32].

In our patients, all *NPHS1* mutations generated a severe NS phenotype of the Finnish type, as previously reported [33–37]. We did not observe the milder phenotype described by others [10, 37].

The second most frequent cause of CNS in our population was *LAMB2* mutations, which are considered to be a rare cause of SRNS, observed in 2.5% of patients in the series by Hinkes et al. [36]. To date, 38 different mutations in the *LAMB2* gene have been described [19]; we extend the spectrum of mutations by reporting two novel mutations, detected in two families, that cause a very severe form of CNS.

The novel nonsense mutation p.E705fsX, which predicts complete loss of function of the laminin-β2 protein, was detected at the homozygous state in six children of family FV. It was characterized by in utero NS development. The renal histopathological lesion was DMS, which was associated with early onset renal failure and rapid death with a median age of 13.8 days (range 0–30 days). We noted the absence of distinct ocular anomalies of Pierson syndrome such as microcoria, abnormal lens shape with cataracts, and retinal abnormalities, usually associated with truncating mutations [19]. Exophthalmia was the only ocular change. However, another extra renal abnormality, facial dysmorphy with asymmetric lying ears, was present.

This disorder can be classified as a severe form of CNS with minor ocular abnormalities, in contrast with previous findings, which speculated that truncating mutations were associated with the complete Pierson syndrome.

Family IV patients bear compound heterozygous mutations associating a maternally inherited frameshift insertion generating a truncated protein (p.D1151fsX23) and a paternally inherited missense mutation (p.S179F), which has already been reported [27]. Clinically, patients carrying these mutations developed a classic phenotype of Pierson syndrome, with early onset nephrosis associated with DMS, abnormalities of the cornea, and rapid death within the first year of life, although this early death was due to intercurrent complications preventing knowledge of what would have been the renal outcome. It is thus not possible to ascertain whether these patients presented with a severe renal phenotype or with a milder form, as has been reported in patients with nontruncating mutations, at least on one allele [19].

Mutations in the *NPHS2* gene have been reported to account for a significant proportion of all nephrotic patients. They correspond roughly to 45–55% of familial forms and 8–20% of sporadic disease, with variations according to the different patient cohorts and the different sub-phenotypes studied [38]. The incidence of familial podocin mutations in our population represented only 7.6%, with childhood onset NS, similar to that in Libyan and Moroccan families (7.6%) [39], lower than in Turkish (29.2%) [40], European, and American children (40%) [41], but higher than in African–American [42], Japanese, and Korean children (0%) [34, 43].

We reported a novel p.E371fsX16 podocin-truncating mutation, associated with the variant p.V180M in one nonconsanguineous family. In the presence of the latter variant, as observed by Weber et al. [11], the occurrence of the disease was delayed, suggesting that podocin might have retained some function in these patients. ESRF was reached at the median age of 11.5 years. The p.V180M, in its homozygous state, was also reported in a Libyan family with the same phenotype [39]. This suggested that the p.V180M variant might be among the frequent mutations detected in North African patients.

The last mutation detected in our patients, was the heterozygous *WT1* mutation, p.R366H in exon 8, affecting one family. This mutation has been previously described [28]. It results in a change in zinc finger 2, affecting the DNA-binding stability of WT1 to the target genes [44]. Early onset NS and DMS, were present in our patient, as described previously in patients with DDS [28, 45, 46]. Our patient was female and no genital or urinary malformations were described.

Mutations in the *NPHS1*, *NPHS2*, and *PLCE1* genes were described to be responsible for most of the severe cases of congenital and early onset NS [6]. In our study no *PLCE1*, but *LAMB2* and *WT1* causing mutations were detected confirming that *LAMB2* and *WT1* screening has to be included in mutation analysis of CNS and infantile NS, if any ocular symptoms are present for the *LAMB2* screening.

It is noteworthy in our series, that 9 out of 24 patients failed to be categorized by mutational analysis. They tended to reach ESRD (median age 10 years) later than children with the mutation. The whole histopathological findings were FSGS. It can be expected that further genetic studies in those families will lead to the identification of new causative genes for childhood-onset SRNS. Indeed, a whole genome-wide linkage analysis in family FXIII was planned. It has been expected that a new genetic defect will be found in this family in the near future.

Therapeutically, the goals of the symptomatic therapy during the first months are to control edema and possible uremia, prevent and treat complications such as infections and thromboses, and provide optimal nutrition [38]. Unfortunately, we noted a higher rate of mortality in our patients with CNS (41.6%) than in central European children. This can be explained by the absence of rapid kidney transplantation, which represents in most cases the only curative treatment.

The limitation of our study is the size of our cohort. Nevertheless, NS is a rare disease and it is rational thus expected to find a low number of patients in a small country such as Tunisia. On the other hand we consider in our study a highly selected cohort of patients and not a population-based sample. Therefore, the frequency that we reported concerns our study group and not the general population. Although the small size of our cohort does not allow recommendations with regard to a diagnostic algorithm, we suggest that screening for *LAMB2* mutations might be useful in patients with CNS if any ocular anomaly is present.

For more general conclusions, our results need to be confirmed in a larger sample of patients.

Conclusion

Our study is to our knowledge the first to describe genetic forms of NS in the Tunisian population. We reported five novel mutations in the *LAMB2*, *NPHS1*, and *NPHS2* genes. CNS and infantile NS, as monogenic diseases, can be explained by mutations in the genes *NPHS1*, *NPHS2*, *WT1*, or *LAMB2*; thus, there is a necessity to include not only the screening of *NPHS1*, but also *NPHS2* and *WT1* in the mutation analysis of CNS, as well as of *LAMB2*, if any ocular anomaly is present. The identification of additional genes as mutated in SRNS is anticipated.

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