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Prevalence of *WT1* mutations in a large cohort of patients with steroid-resistant and steroid-sensitive nephrotic syndrome

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Prevalence of *WT1* mutations in a large cohort of patients with steroid-resistant and steroid-sensitive nephrotic syndrome.

Background. Nephrotic syndrome (NS) represents the association of proteinuria, hypoalbuminemia, edema, and hyperlipidemia. Steroid-resistant nephrotic syndrome (SRNS) is defined by primary resistance to standard steroid therapy. It remains one of the most intractable causes for end-stage renal disease (ESRD) in the first two decades of life. Sporadic mutations in the Wilms' tumor suppressor gene *WT1* have been found to be present in patients with SRNS in association with Wilms' tumor (WT) and urinary or genital malformations, as well as in patients with isolated SRNS.

Methods. To further evaluate the incidence of *WT1* mutations in patients with NS we performed mutational analysis in 115 sporadic cases of SRNS and in 110 sporadic cases of steroid-sensitive nephrotic syndrome (SSNS) as a control group. Sixty out of 115 (52%) patients with sporadic SRNS were male, 55/115 (48%) were female. Sex genotype was verified by haplotype analysis. Mutational analysis was performed by direct sequencing and by denaturing high-performance liquid chromatography (DHPLC).

Results. Mutations in *WT1* were found in 3/60 (5%) male (sex genotype) cases and 5/55 (9%) female (sex genotype) cases of sporadic SRNS, and 0/110 (0%) sporadic cases of SSNS. One out of five female patients with mutations in *WT1* developed a WT, 2/3 male patients presented with the association of urinary and genital malformations, 1/3 male patients presented with sexual reversal (female phenotype) and bilateral gonadoblastoma, and 4/5 female patients presented with isolated SRNS.

Conclusion. According to the data acquired in this study, patients presenting with a female phenotype and SRNS and male patients presenting with genital abnormalities should especially be screened to take advantage of the important genetic information on potential Wilms' tumor risk and differential therapy. This will also help to provide more data on the phenotype/genotype correlation in this patient population.

Key words: *WT1*, nephrotic syndrome, genital malformations.

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Nephrotic syndrome (NS) is defined as the association of proteinuria, hypoalbuminemia, edema, and hyperlipidemia. It constitutes one of the most common diagnoses made in pediatric nephrology. About 80% of all children with sporadic NS respond to steroid treatment. For decades, NS has been separated into two broad categories based upon its response to standard steroid therapy: steroid-*sensitive* nephrotic syndrome (SSNS) versus steroid-*resistant* nephrotic syndrome (SRNS) [1, 2]. In SRNS, about 75% of patients exhibit renal histology of focal segmental glomerulosclerosis (FSGS), and about 20% exhibit renal histology of minimal change nephrotic syndrome (MCNS). In SSNS, renal histology reveals MCNS in 80% and FSGS in 20% [3]. Positional cloning revealed defects in four different genes as monogenic causes for familial cases of SRNS. Mutations in *NPHS1*, encoding nephrin (OMIM 602716) cause congenital nephrotic syndrome (CNS) of the Finnish type [4]. *NPHS2* (podocin) mutations (OMIM 604766) cause SRNS type 1 [5]. Mutations in *ACTN4* encoding a-actinin 4 (OMIM 604638) have been identified as an autosomal-dominant cause of SRNS [6]. An additional locus has been mapped to chromosome 11q21-q22 for an autosomal-dominant form of nephrotic syndrome (OMIM 603965) [7]. Gene identification underlines the importance of genetic factors in the pathogenesis of NS. Through identification of these three genes as causative for SRNS, their gene products, nephrin, podocin, and α-actinin 4, were identified as important for the function of the glomerular podocyte [8].

Furthermore, sporadic mutations in the tumor suppressor gene *WT1* have been found to be present in patients with SRNS, providing the fifth genetic cause of NS [9–11]. *WT1* encodes a transcription factor of the zinc finger family. Four different transcripts are produced by alternative splicing [12, 13]. *WT1* was identified by positional cloning aiming to identify the gene responsible for the childhood cancer, Wilms' tumor (WT) [14, 15]. Intensive investigation afforded to *WT1* has revealed the association of *WT1* mutations with Denys-Drash syndrome (DDS) [16, 17] and the Frasier syndrome (FS) [18]. DDS in 46, XY patients present as a triad of WT, XY pseudohermaphroditism, and progressive glomerulopathy; 46, XX patients present with WT and progressive glomerulopathy, with absence of XY pseudohermaphroditism [9–11, 19–22]. Incomplete forms of DDS have been described, including 46, XY patients with isolated diffuse mesangial sclerosis [10, 22–24]. FS has been described as the association of complete XY gonadal dysgenesis, NS, and the development of gonadoblastoma in 46, XY patients, and as isolated nephropathy in 46, XX patients [19, 21]. Patients with DDS show gomerulopathy characterized mostly by the histologic finding of diffuse mesangial sclerosis (DMS); the predominant lesion in patients with FS is FSGS. In DDS, the nephropathy occurs in infancy and usually progresses to end stage renal disease (ESRD) in less than three years, whereas in FS, it occurs later and leads to ESRD in the second decade of life [9, 19]. The risk for the development of a WT seems to be increased predominantly in DDS [19]. In patients with genetic characteristics of FS, an association with WT has also been described [23, 25, 26]. DDS and FS form an overlapping spectrum of disease presentation. Patients sharing the clinical and genetic characteristics of both disorders have been described. Constitutional heterozygous germline mutations of *WT1* have been identified in most of the patients with DDS and FS [22]. In patients with DDS over 95% of the mutations are found as missense mutations in exon 8 and 9 of the *WT1* gene, encoding for zinc finger 2 and 3 [10]. FS is associated with specific splice site mutations in exon 9 [24]. Female patients with mutations in *WT1* mostly present with isolated nephropathy or nephropathy in association with WT. However, 46, XX patients with *WT1* mutations with urinary and genital malformations have been described [10]. To further evaluate the incidence of *WT1* mutations in patients with NS, we performed mutational analysis in 115 sporadic cases of SRNS, and in 110 sporadic cases of SSNS as a control group.

METHODS

Blood samples for mutational analysis, clinical data, and informed consent (www.renalgenes.org) were obtained from patients or their parents. Genomic DNA was isolated directly from blood samples by standard methods [27]. Diagnoses of SRNS and SSNS were established by a pediatric nephrologist according to published criteria [1]. For clinical evaluation we used a standard questionnaire (www.renalgenes.org) as previously described [28]. Characteristic features defining the clinical diagnosis were: age of onset of nephrotic syndrome, response to steroid therapy, histology of the kidney biopsy, progression to ESRD, the recurrence of FSGS after renal transplantation, and the association with extrarenal manifestations. Standard steroid treatment and response to steroid treatment, as well as response to cyclosporine A (CyA) and cyclophosphamide (CP) therapy, were defined according to the International Study of Kidney Disease in Children (ISKDC) and Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN) guidelines [1, 2]. Patients were categorized steroid-sensitive if at least a partial response to steroids was present. Patients turning steroid-resistant in a later stage of the disease were considered as "steroid-sensitive" (SSNS) for this study. Congenital nephrotic syndrome (CNS) was defined as the presentation of NS within the first two months of life. Ethnic backgrounds of patients were central Europe, Turkey, and India. Before performing mutational analysis in exon 6–9 of *WT1*, mutations in *NPHS2* (podocin) were excluded. One hundred fifteen sporadic patients with SRNS and 110 sporadic patients with SSNS were included in the study. Sixty out of one hundred fifteen (52%) patients with SRNS were male (sex genotype), 55/115 (48%) were female (sex genotype). The sex genotype of the patients was verified by performing polymerase chain reaction (PCR) with markers *DXS9902*, *GATA172D05*, and *DYS390*. Except for one patient (F1194), sex genotype and sex phenotype were identical for all patients with SRNS included in this study. Haplotype analysis was performed on an ABI Prism® 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as described elsewhere [29]. The clinical data were as follows. Renal biopsy results in patients with sporadic SRNS were: FSGS 73/115 (63%), MCNS 17/115 (15%), mesangioproliferative glomerulonephritis (MP) 3/115 (3%), diffuse mesangial sclerosis (DMS) 3/115 (3%), membranoproliferative glomerulonephritis (MPGN) 5/115 (4%), no data or no biopsy performed 14/115 (12%). In 66 of 110 patients with SSNS, no renal biopsy was performed. In the remaining 44 patients with SSNS, biopsy results were as follows: FSGS 20/44 (45%), MCNS 17/44 (39%), MPGN 5/44 (11%), MP 2/44 (5%) (Table 1). The low number of biopsies performed in SSNS is explained by the milder course of SSNS. The median age of onset was 4.8 years in the SRNS patients and 4.3 years in the SSNS patients (Table 1). CNS was diagnosed in 5/115 (4%) patients with SRNS. The median age of onset of NS for the SRNS patients was 5.0 after subtracting the CNS patients (Table 1). Thirty-two of 115 (28%) of the SRNS patients progressed

Table 1. Clinical and mutational analysis data in 115 SRNS patients and 110 SSNS patients

Group	Number of patients	Median age of onset *years*	Biopsy FSGS/ MCNS/other/ND %	ESRD	Median age at ESRD *years*	Male/female relation
SRNS						
WT1 mutation	8/115[a]	4.4[b]	7/0/0/1	3 (38%)	13.0	3/5
Absence of *WT1* mutation	107/115[c]	4.9[d]	66/17/11/13	29 (27%)	11.0[e]	57/50
Total of SRNS patients	115[f]	4.8[g]	73/17/11/14	32 (28%)	11.2[h]	60/55
SSNS						
WT1 mutation	0/110	NA	NA	NA	NA	NA
Absence of *WT1* mutation	110/110	4.3	20/17/7/66	3 (3%)	14.9	NA
Total of SSNS patients	110	4.3	20/17/7/66	NA	NA	NA

Abbreviations are: SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; CNS, congential nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; MCNS, minimal change nephrotic syndrome; ESRD, end-stage renal disease; ND, no data available; NA, not applicable.
[a]One patient presented as CNS; [b]median was 4.5 after subtracting the CNS patient; [c]4 patients presented as CNS; [d]median was 5.0 after subtracting the 4 CNS patients; [e]median was 12.4 after subtracting 1 CNS patient; [f]5 patients presented as CNS; [g]median was 5.0 after subtracting the 5 CNS patients; [h]median was 12.5 after subtracting the CNS patients with ESRD.

Table 2. Primer sequences used for mutational analysis

Exon	Forward	Reverse
6	CCATCATTCCCTCCT GATTG	AGCCTGCAGTGAAG AAGAGG
7	AAGACCTACGTGAAT GTTCAC	GTGTGAGAGCCTGG AAAAGG
8	CCTTTAATGAGATCCCC TTTTCC	GGGGAAATGTGG GGTGTTTCC
9	CCTCACTGTGCCCACA TTGT	GCACTATTCCTTCTC TCAACTGAG

to ESRD with a median age of 11.0 years (Table 1). Three of 110 SSNS patients progressed to ESRD. Two of 60 (3%) male patients presented with genital or urinary malformations, 1/60 (2%) male patients presented with sexual reversal, and 0/55 (0%) of female patients presented with genital or urinary malformations.

Mutational analysis of exons 6 and 7 of the *WT1* gene was performed by denaturing HPLC (DHPLC), mutational analysis of exons 8 and 9 by direct sequencing of the forward strands. Primers are shown in Table 2. Known single nucleotide polymorphisms within the primer sequence were avoided (http://genome.ucsc.edu/) because known single nucleotide variants within the primer sequence can suppress amplification of one of the two alleles of the amplified product. PCR products of exons 6 and 7 from the patients were pooled with a control individual and subjected to analysis by DHPLC [30]. Melting profiles were determined according to the manufacturer's recommendations (Transgenomic, Inc., Omaha, NE, USA). Column temperatures for DHPLC analysis were 60.9°C, 61.9°C, 62.9°C for exon 6, and 60.7°C, 61.7°C, 62.7°C for exon 7. Direct sequencing was performed for samples containing heteroduplex elution peaks. Sequencing was performed as previously described [29]. Very high sequence quality was obtained. If in doubt, the complementary strand was also sequenced. To rule out polymorphisms, 160 chromosomes of healthy control individuals were checked for novel mutations by direct sequencing. For sequence evaluation the program Sequencher™ (GeneCode, Ann Arbor, MI, USA) was used.

RESULTS

Types of *WT1* mutations found

Mutational analysis of exons 6–9 of *WT1* was performed in 115 sporadic cases of SRNS and 110 patients with SSNS. In 8/115 sporadic cases with SRNS and in none of 110 sporadic SSNS cases were mutations detected in exon 6–9 of *WT1*. Two different missense mutations and 6 splice site mutations were identified. These two missense mutations identified in exon 9 were novel (Table 3, Fig. 1). Both novel mutations were absent in 160 chromosomes of healthy control individuals. They consisted of a T1162C transition leading to a nonconservative amino acid exchange C388R, found heterozygously in F1031. A similar mutation C388Y affecting the same codon was described before [31]. An A1190C transition leading to a nonconservative amino acid exchange H397P was found heterozygously in F734 (Fig. 1). The known splice site mutation IVS9+4 G > A was found heterozygously in F953, F999, and F1194. The known splice site mutation IVS9+5 G > A was found in F921, F963, and F1073 (Table 3). Thirty patients with sporadic SRNS and mutations in *NPHS2* (podocin) [32] were also analyzed for mutations in *WT1*. No mutation in exons 6–9 of *WT1* could be identified in these patients. The 8 patients carrying mutations in *WT1* were examined for mutations in the 8 exons of *NPHS2*. In none of these patients was a mutation in NPHS2 identified.

Clinical data

In 8 SRNS patients mutations in exons 6–9 of *WT1* were identified. Missense mutations in exon 8 and 9 are known to be associated with DDS. Patients F1031 (C388R) and F734 (H397P) with missense mutations in exon 9 presented with early onset of NS. Histology of the kidney biopsy revealed FSGS in F734, no biopsy was performed

Table 3. Clinical data of patients with mutations in WT1

Family	Sex genotype	Sex phenotype	Mutation	Age of onset years	Initial symptoms	Biopsy	Steroid therapy	CP/CyA therapy	ESRD *years after AO*	KTx *years after AO*	Relapse FSGS *years after KTx*	Age at diagnosis of Wt *years*	Genital status/ extrarenal abnormalities
921	f	f	Ex9 IVS9+5 G/A (h)	2.3	A	FSGS	SR	CP (NR)	Y(5.8)	Y(6.7)	N(11.5)	N	N
953	f	f	Ex9 IVS9+4 C/T (h)	5.0	A	FSGS	SR	nd	N(2.5)	N	N	N	N
963	m	m	Ex9 IVS9+5 G/A (h)	4.5	?	FSGS	?	?	Y(11)	N	N	N	test. atrophy/microcalcification
999	f	f	Ex9 IVS9+4 C/T (h)	6.9	A	FSGS	SR	CP (NR)	Y (6.1)	Y (13.4)	N (CR 6.0)	N	N
1073	m	m	Ex9 IVS9+5 G/A (h)	8.9	A	FSGS	SR	CyA (NR) CP (R)	N (0.9)	N	N N	N	Hypospadias
1194	m	f	Ex9 IVS9+4 C/T (h)	4.2	A	FSGS	SR	CP (NR)	N	N	N	N	Sexual reversal/gonadoblastoma
734	f	f	Ex9 A1190C/H397P (h)	1.1	A	FSGS	SR	CyA (PR)	N (0.5)	N	N	N	N
1031	f	f	Ex9T1162C/C388R (h)	0.1	A	nd	nd	nd	N (0.5)	N	N	Y (1.5)	N

Abbreviations are: A, acute onset of nephrotic syndrome with edema; AO, age of onset of nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; CP, cyclophosphamide; CyA, cyclosporine A; CR, chronic rejection; Ex, exon; ESRD, end-stage renal disease; f, female; h, heterozygous; KTx, kidney transplantation; m, male; N, No; nd, not done; NR, no remission; PR, partial remission; R, remission; SR, steroid-resistant; test, testicular, WT, Wilms' tumor, Y, yes; ?, no data.

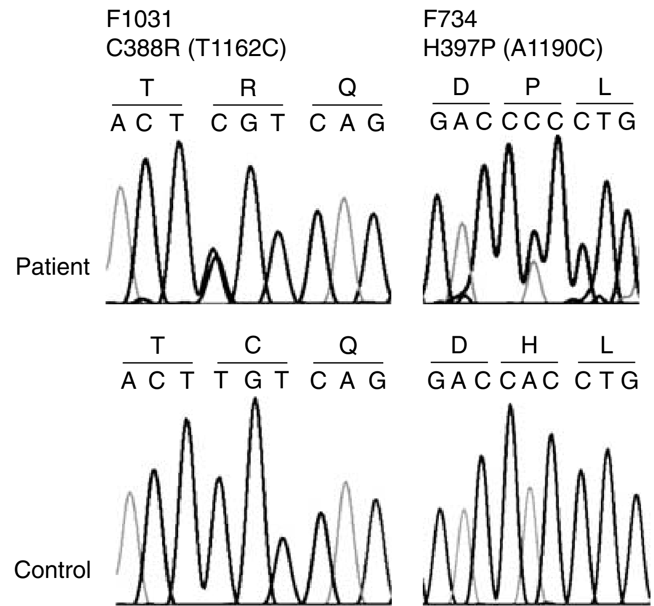

Fig. 1. Detection of two novel *WT1* mutations by direct sequencing in F734 and F1031. The corresponding nucleotide sequences with the encoded amino acids are shown above each chromatogram. The upper chromatograms show the novel heterozygous missense mutations C388R (T1162C) (*left*) and H397P (A1190C) (*right*) in *WT1*. The lower chromatograms show a healthy control individual.

in F1031 (Table 3). F1031 developed a unilateral WT at the age of 1.5 years. As expected, genital or urinary malformations were not described for any of the female patients with a missense mutation in *WT1*.

Splice site mutations in exons 8 and 9 of *WT1* are known to be associated with FS. The onset of NS in the 6 patients with splice site mutations was later compared to the patients with missense mutations with an average of 5.3 years (range 2.3 to 8.9). Patients F921, F963, and F999 progressed to ESRD with an average of 7.6 years (range 5.8 to 11 years). These data are consistent with previously published data [9, 10]. Kidney biopsies showed FSGS in all patients. Patient F999 and F921 received a kidney transplant. No recurrence of FSGS in the transplant was described. In the male patient F963, nephropathy was associated with a bilateral testicular atrophy, in the male patient F1073, the nephropathy was associated with hypospadias. For all patients with SRNS the sexual genotype was verified by haplotype analysis with markers *DXS9902*, *GATA172D05*, and *DYS390*. Patient F1194 with a female phenotype showed a XY karyotype, which corresponds to a complete sexual reversal. After identifying a splice site mutation in exon 9 and verifying the karyotype in this patient, gonadectomy was performed. Histology revealed a bilateral gonadoblastoma. No signs of metastatic spread were present. This underlines the importance of mutational analysis in these patients. Except for F1194, sex genotype and sex phenotype were identical for all patients with SRNS included in this study. No

genital or urinary malformations were described in the female patients (Table 3).

DISCUSSION

Here we describe the results of the first screening for mutations in exons 6–9 of *WT1* in a large cohort of patients with SRNS. In 8/115 (7%) of sporadic SRNS cases mutations in exons 6–9 of *WT1* were identified altogether. In two female patients, missense mutations in exon 9 consistent with the diagnosis of DDS were identified; in one of them WT was diagnosed. In three male and three female patients, splice site mutations in exon 9 consistent with the diagnosis of FS were found. Two of the male patients presented with urinary or genital malformations, one of them with sexual reversal and bilateral gonadoblastoma. All three female patients presented with isolated FSGS. No mutations were found in 110 sporadic cases of SSNS, used as a control group. Our study generates the first data about the occurrence of *WT1* mutations in a large cohort of sporadic SRNS patients.

Two different clinical phenotypes associated with *WT1* mutations and glomerulopathy have been described in the literature. Missense mutations most commonly found in exons 8 and 9 are associated with DDS, and specific splice site mutations in exon 9 with FS. However, patients with characteristic genetic findings of FS presenting with clinical symptoms of DDS and vice versa were described [10, 21, 23, 25, 26], suggesting that both diseases should be considered as part of a spectrum of *WT1* gene mutations rather than as separate diseases.

Here we describe two novel missense mutations leading to amino acid exchanges C388R and H397P. According to the literature these findings are consistent with the diagnosis of DDS [10]. The mutations were absent in the parents of F1031 and the mother of F734, giving evidence for a spontaneous mutation in both patients. Early onset of NS was present in both patients as described previously for patients with DDS by others [9–11]. F1031 also developed a unilateral WT. As both patients were female, no genital or urinary malformations were described. In contrast to the classic form of DDS, the renal histology of patient F734 revealed FSGS. Other cases with missense mutations and the diagnosis of FSGS have been described before [9]. This further emphasizes the clinical overlap of DDS and FS. The occurrence of the WT in F1031 underlines the importance of the *WT1* screening in patients with early onset NS. The finding of missense mutations in *WT1* indicates an increased risk of WT; therefore, careful monitoring of all patients with *WT1* mutations is required. After reaching ESRD, bilateral nephrectomy should be performed to prevent the development of a WT.

In 6 patients splice site mutations in exon 9 were identified, consistent with the diagnosis of FS [24]. These mutations impair the use of the second alternative splice donor site. Normally, alternative splicing results either in the inclusion or exclusion of the three amino acids lysine, threonine, and serine (KTS) in a ratio of approximately 2:1 +KTS/−KTS isoforms. Splice site mutations in exon 9 lead to a reversal of this +KTS/−KTS isoform ratio to 1:2. The presence of both alternatively spliced isoforms is crucial for normal kidney development. The reduction of the +KTS levels lead to the development of glomerulosclerosis [33]. Typically, the onset of NS and the progression to ESRD was later in our patients presenting with FS, compared with DDS. The renal histology was FSGS in all patients. Second, the +KTS/−KTS isoforms play an important role in male sexual differentiation by regulating the Y chromosome–encoded Sry gene, which initiates male sex determination in mammals. Mice lacking the +KTS isoform were shown to have significantly reduced Sry levels, and hence, explain the lack of the initiation of the male sex determination pathway in these animals [33]. Nephropathy was associated with genital and urinary malformations in the male patients, F1073 presented with hypospadias, and F963 with testicular atrophy and testicular microcalcification. In F1194 a 46, XY karyotype with sexual reversal was found. Because of the increased risk of gonadoblastoma, gonadectomy was performed and bilateral gonadoblastoma without metastatic spread was diagnosed, again emphasizing the importance of the screening and of the verification of the karyotype.

46, XY patients with DDS present with a broad spectrum of intersex and hermaphrodite phenotypes; FS patients with a 46, XY karyotype can present with normal female external genitalia, and those with a 46, XX karyotype appear to have normal gonadal development. As shown in F1194, sex reversal in 46, XY patients is associated with streak gonads and a high risk of gonadoblastoma; the karyotype should be examined in all female patients carrying mutations in the splice site of exon 9. In our study genital or urinary malformations were absent in 5 female patients with mutations in *WT1*. Patients F963 and F1073, carrying a characteristic mutation for FS only, show impairment of the genital and urinary development but still present with male external genitalia. This further underlines the complexity of the phenotypes seen in patients with mutations in *WT1*. WT was diagnosed in one patient with characteristic mutation for DDS as compared to none of the patients with mutations characteristic for FS. Previously, the absence of WT was considered as a characteristic feature of FS compared to the high incidence of WT in DDS. Nevertheless, recently, FS cases carrying the characteristic splice site mutation and presenting with WT were reported [23, 25, 26]. Although specific characteristics apply to FS and DDS, many questions on the phenotype/genotype correlation remain unanswered.

In the initially published studies of *WT1* mutational analysis, more patients associated with WT or urinary or

genital malformations were included. This explains the previously published male/female ratio of mutations in WT1 of approximately 2:1 [9]. It was suggested that in the past the correct diagnosis had been missed in female patients because female patients in general may have less severe or missing gonadal problems, when carrying mutations in *WT1* [9]. In the meantime, different studies have been performed on mutational analysis in single cases, as well as in smaller cohorts of isolated FSGS or isolated DMS. Thereby, it was shown that mutations in *WT1* show a common cause of isolated FSGS in female patients [9–11, 19]. Here we describe the first data on the prevalence of mutations in a large cohort of patients with SRNS. We identified mutations in exons 6–9 of *WT1* in 5/55 (9%) female patients with sporadic SRNS and in 3/60 (5%) male patients with sporadic SRNS. In contrast to the previously published data, the incidence of mutations in female patients with isolated FSGS is surprisingly high. The lower incidence of mutations in *WT1* in male patients compared to the female patients can be explained by the fact that only 2/59 phenotypical male patients with urinary or genital malformations were included in this study.

CONCLUSION

The data provided in this study on mutational analysis in 115 sporadic patients with SRNS emphasize the relevance of mutational analysis of *WT1* in this cohort. The data generated here present clear evidence for the importance of extending the screening of *WT1* mutations to all children with SRNS. In patients with *WT1* mutations, a renal ultrasound screening for the development of tumors should be employed. According to the data acquired in this study, especially patients presenting with a female phenotype and SRNS, and male patients presenting with genital abnormalities, should be screened. In male and female patients with DDS there is a high risk for the occurrence of WT. Therefore, close renal ultrasound monitoring should be applied in all patients. When reaching ESRD, nephrectomy should be performed to prevent WT. In male patients with FS and sexual reversal, the incidence for gonadoblastoma is increased; again, close ultrasound monitoring and gonadectomy should be performed in these cases. Although the risk for WT is much lower in FS than in DDS, the occurrence of WT should be still considered in FS, as described for previously published cases. In addition, future studies may relate the prognosis on the outcome of kidney transplantation, as well as the efficiency of immunosuppressive therapy to the presence of *WT1* mutations. For this reason, mutational analysis should be extended to all children presenting with SRNS to take advantage of the very important genetic information when treating these children. This will also help to provide more data on the phenotype/genotype correlation in this patient population.

APPENDIX

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