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Spectrum of early onset nephrotic syndrome associated with *WT1* missense mutations

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Spectrum of early onset nephrotic syndrome associated with *WT1* missense mutations. We investigated 17 children with nephrotic syndrome (NS) of early onset (14 aged < 1 year) and rapid progression to end-stage renal disease for the presence of mutations in the Wilms' tumor suppressor gene *WT1* on chromosome 11. In eight children (7 genotypic males) an association with Wilms' tumor and/or ambiguous genitalia (Denys-Drash syndrome) was observed. In these eight and two additional female patients with NS only constitutional missense mutations in the *WT1* gene were detected; four children presented the so-called hot spot mutation in exon 9 (R394N) and six had different mutations in exons 8 and 9 (4 not previously described). Renal biopsy showed diffuse mesangial sclerosis in eight and focal segmental sclerosis in two cases. End-stage renal disease was reached either concomitantly or within four months after onset of NS in seven of ten patients. A unilateral Wilms' tumor was found before or concomitant with NS in four children (3 males, 1 female). From the seven genotypic males with *WT1* mutations, five presented ambiguous genitalia and two a female phenotype. No mutation of the *WT1* gene was found in seven other children with isolated congenital or infantile NS with or without DMS who appeared to have a slower progression than the first group. It is proposed that patients with early onset, rapidly progressive NS and diffuse mesangial or focal segmental sclerosis should be tested for *WT1* mutations to identify those at risk for developing Wilms' tumor.

The triad of Wilms' tumor (WT), male pseudohermaphroditism and progressive glomerulopathy is known as Denys-Drash syndrome (DDS) [1, 2]. Subsequent reports described patients with incomplete forms of this syndrome [3–5] dividing DDS into three clinical categories: (*1*) genotypic males with all three abnormalities, (*2*) genotypic males with nephropathy and ambiguous external and/or internal genitalia only, and (*3*) genotypic females with nephropathy and WT only. All patients show glomerulopathy, usually characterized by the histologic finding of diffuse mesangial sclerosis (DMS) and in rare cases focal segmental glomerulosclerosis (FSGS) is observed [3, 4]. DMS may also be observed as an isolated sporadic or familial disorder [6]. Clinically DMS is characterized by massive proteinuria with a nephrotic syndrome starting early in life and progressing rapidly to end-stage renal disease (ESRD) [3, 4, 6]. The only difference between isolated DMS and DMS associated with DDS seems to be the familial occurrence in the former. WT associated with DDS is more often bilateral and on the average has an earlier onset than isolated (sporadic) WT.

WT1, one of the WT suppressor genes was isolated by positional cloning from chromosome 11p13 [7, 8] and encodes a transcription factor of the zinc finger (ZF) family. Four transcripts are produced by alternative splicing [9, 10]. In WT patients a variety of mutations in the *WT1* gene was described [11]. The *WT1* gene plays a specific role during the embryonic development of the kidney, genitalia and other organs [12–15]. Mice with a homozygous deletion of *WT1* fail to activate the development of the urogenital system, confirming its key role in this process [16]. The expression of *WT1* in both kidney and urogenital system might explain that DDS is often associated with a mutation in this gene [13]. Our review of the literature [17–35; summaries, 11, 35] showed that among 50 patients with complete or incomplete forms of DDS 21 different *WT1* mutations were found. Most patients presented the characteristics of DMS as described by Habib et al [3, 6].

Attempts to correlate the phenotype of DDS and different *WT1* mutations did not provide clearcut results. However, these investigations were often hampered by insufficient accounts of the clinical and pathoanatomical manifestations and the lack of reporting comparable patients without *WT1* mutations.

The primary aims of this work was (*1*) to identify mutations of the *WT1* gene in a large spectrum of patients with progressive forms of early onset NS with or without the clinical diagnosis of DDS; (*2*) to correlate the different *WT1* mutations with the phenotype of DDS; and (*3*) to identify patients with an increased

Key words: nephrotic syndrome, Denys-Drash syndrome, renal failure, Wilms' tumor, diffuse mesangial sclerosis.

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risk to develop WT. For this study infants with complete or incomplete DDS were investigated and patients of the same age range with isolated NS associated with DMS or other renal histopathology. In addition, we searched for *WT1* mutations in some relatives.

METHODS

Patients

The clinical and pathoanatomic features of 17 children with early onset NS studied are summarized in Table 1. Three genotypic male children had the complete form of DDS and four males an incomplete form without WT. Five of the seven males had presented with a NS in the first year of life, corresponding to a congenital or infantile NS; two had an onset of NS at three years of age. One of the three females presented with NS at 18 months, a WT being found concomitantly (NS8).

In addition, we investigated one male and three females with isolated congenital or infantile NS in absence of a WT or obvious genital anomalies associated with DMS (NS9-12) and five children with a similar age at onset but different renal histopathology. Five of the 17 patients had a family history of progressive NS (NS11, NS14-NS17) and five had more or less severe extrarenal/extragenital malformations which were missing in the group with *WT1* mutations. All 17 except one patient (NS16) developed ESRD; most were treated with dialysis and subsequently underwent transplantation. Three patients died (Table 1). Patients with congenital NS of the Finnish type were excluded from this study.

Molecular methods

Mutations of the *WT1* gene were searched for in blood DNA from the 17 patients listed in Table 1 and from some asymptomatic relatives (6 parents, 2 siblings) in four affected families. In addition, we investigated tumor DNA from one patient obtained at the time of nephrectomy.

Preparation of genomic DNA. Constitutional DNA was isolated from blood samples (5 ml) by the SDS-proteinase K method as described previously [36].

PCR-SSCP analysis of the WT1 gene. We used the single-strand conformational polymorphism (SSCP) method to analyze all *WT1* coding exons for alterations within the germline of patients [37]. All 10 exons were amplified by the polymerase chain reaction (PCR) from genomic DNA.

Polymerase chain reaction. The sequences of the oligonucleotides were as published [36]. Each PCR reaction contained 100 ng genomic DNA, 1x *Taq* DNA Polymerase buffer, 25 pmol of each primer, 200 μM nucleotides and 1 U *Taq* Polymerase in a total volume of 50 μl. Conditions for the different PCRs were as described previously [36].

Single-strand conformational polymorphism. Following PCR a 1 to 1.5 μl aliquot of the amplified product was diluted with 4 to 4.5 μl 95% formamide, 10 mM EDTA. The DNA samples were denatured at 95°C for 10 minutes and immediatly placed on ice before loading on 8% polyacrylamide nondenaturing gels (29:1 or 49:1 acrylamide:bisacrylamide) containing 2% or 10% glycerol. Electrophoresis was performed in 0.09 M Tris base-0.09 M boric acid-2 mM EDTA running buffer at 500 Volt at 15°C for two to four hours. The gels were then silver stained. Every sample was analyzed using four sets of electrophoresis conditions to maximize the sensitivity of the technique.

Restriction enzyme analysis. Restriction digests were performed overnight according to the manufacturers instructions. The DNA fragments were separated on 3% NuSieve agarose gels and visualized with ethidium bromide staining.

Direct sequencing of PCR products. Single-stranded sequence templates were prepared using biotinylated primers and streptavidin coated magnetic beads (Dynal, Norway) according to the manufacturers recommendation. Dideoxy sequencing was performed using the Sequenase Kit Version 2.0 (US Biochemicals, USA) and ^{35}S-ATP labeling. The products were separated on 6% denaturing polyacrylamide gels and exposed to X-ray film overnight. In one case direct sequencing was performed using the automated LI-COR sequencer (MWG Biotech).

RESULTS

In 10 of the 17 patients, mutations in the *WT1* gene were found. Four mutations have not been described before (Table 1). All patients were analyzed for mutations in the *WT1* gene using the SSCP method. First the four exons encoding the four zinc fingers were analyzed and when no alterations were found the rest of the gene was analyzed as well. Alterations detected by SSCP were sequenced and verified if possible by a restriction enzyme digest. DNAs with known mutations were loaded on the same gel to check for the presence of these mutations.

The hot spot mutation 394 Arg>Trp in exon 9 (now called R394W) was found in four patients. Figure 1 shows the SSCP analysis of three patients and one normal control (NEK). Two additional bands are seen in the DNA from patients NS1, NS2 and NS8, when compared to normal NEK DNA. Tumor DNA was analyzed from patient NS8 and it shows only the altered bands, demonstrating that the tumor has lost the normal allele. The mutation abolishes a *Hpa*II restriction enzyme site, and can therefore be detected by digestion of the PCR products with this enzyme (Fig. 2). Both, the mutant (undigested) and wild-type (digested) alleles are seen in blood DNA from patients NS1, NS2 and NS8. This hot spot mutation was observed in two genotypic males (NS1 and NS2) with complete DDS and one female (NS8) with incomplete DDS. In addition, it was found in a female (NS10) with isolated infantile NS, suggesting that this is also a DDS patient (data not shown).

Three other patients exhibited different mutations in *exon 9*: (*1*) a boy (NS5) with incomplete DDS and a new mutation at the hot spot location, changing nucleotide 1181 G>A and amino acid 394Arg to Gln, characterized by a very rapid course to ESRD and FSGS in the renal biopsy; (*2*) a male patient (NS3) with complete DDS and a new mutation at nucleotide position 1153 T>C changing amino acid 385 Cys to Arg (Fig. 3); (*3*) a male patient NS4 with incomplete DDS having ambiguous external genitalia and no Wilms tumor with a mutation changing an amino acid directly involved in DNA binding (D396N, data not shown).

Three patients had different mutations in *exon 8*: two genotypic males, one with ambiguous (NS6) and one with female genitalia (NS7) but without WT, and a girl with isolated congenital NS and an advanced form of diffuse glomerular sclerosis leading to ESRD at the age of three months (NS9). Patient NS7, who had the most precipitated course in this series, will be reported elsewhere (Guschmann et al, manuscript submitted). Patients NS6 and NS9 have novel mutations at nucleotide position 1090 T>C and 1135 G>T, respectively. The first of these mutations changes an amino acid in the binding backbone of the protein and the second

Table 1. Summary of clinical features and *WT1* mutations in 17 patients with early onset nephrotic syndrome (NS)

Patient	Karyo-type	Genital status/ extrarenal anomalies	Wilms' tumor diagnosis age (*in months*), location, stage, histology	Glomerulopathy *age at onset in months, if not indicated otherwise* Proteinuria *g/m²/day*	NS S_{Alb} *g/l*	RF S_{Cr} *mg/dl*	ESRD	NX	Renal histology	RRT (*age at last observation in years*)	*WT1 mutation* Patient	Relatives examined
Complete DDS												
NS1	46XY	F, uterus, streak gonads	4, R, I, tri	4 (2.2 g/mol Creatinine)	4 (25)	4 (1.1)	20	4 R 20 L	DMS	PD (4.4)	Ex9:1180 C>T R394W	Father- Mother-
NS2	46XY	hypospadias cryptorchidism micropenis	32, R, III, tri	32 60 (4.6)	60 (28)	60 (1.0)	67	32 R	FSGS, IF	HD->TX (10.5)	Ex9:1180 C>T R394W	
NS3	46XY	hypospadias, cryptorchidism micropenis, vagina, uterus, tuba, s. urog., hypoplastic gonads	36, R, I, s	38 (1.2)	38	38 (1.5)	52	37 R 74 L	DMS, IF	PD->TX (11.0)	Ex9:1153 T>C C385R[e]	
Incomplete DDS												
NS4	46XY	large clitoris and labia majora	—	9	9	9 (acute)	9	26 R+L	DMS	PD->TX (3.6)	Ex9:1186 G>A D396N	Father- Mother- AC-
NS5	46XY	hypospadias, cryptorchidism micropenis	—	4d (0.4)	4d (20)	4d (0.8)	4	—	FSGS, IF	PD (3.4)	Ex9:1181 G>A R394Q[e]	
NS6	46XY	hypospadias, cryptorchidism vagina, uterus, tuba	—	6 (4.2)	6 (20)	6 (1.1)	9	—	DMS, IF	died at 9 mo no RRT	Ex8:1090 T>C F364L[e]	
NS7	46XY	F, vagina, cryptorchidism hypoplastic gonads	—	3d (2.0)	3d (20)	3d (2.4)	12d	—	DMS, IF	died at 13 days no RRT	Ex8:1097 G>A R366H	
NS8	46XX	F	18, L, I, tri	18	18 (20)	18 (1.6)	19	19 L 38 R	DMS, IF	PD->TX (5.5)	Ex9:1180 C>T R394W	
Isolated congenital/Infantile NS with DMS (DDS)												
NS9	46XX	ext. F	—	3 (4.5)	3 (15)	3 (0.4)	3	24 L 26 R	DMS, IF	PD->TX (3.6)	Ex8:1135 G>T G379C[e]	Father- Mother- Sister-
NS10	46XX	ext. F	—	6 (1.0 g/dl)	6 (24)	6 (3.2)	6	9 L 14 R	DMS, IF	PD (2.3)	Ex9:1180 C>T R394W	Brother-
Isolated congenital/Infantile NS with DMS												
NS11[a]	46XY	M, psychomotor retardation	—	2 (2.5)	2 (17)	5 (1.9)	5	—	DMS	PD->TX (3.4)	—	
NS12	46XX	F, Rieger syndrome	—	1 day	1 day	1 day	1	—	DMS	PD->RTX died at 1.5 years	—	
Isolated congenital/Infantile NS associated with different renal histopathology												
NS13	46XX	F	—	2	11 (15)	30 (1.0)	36	—	MGN	PD->TX->HD->TX (19.0)	—	
NS14[b]	46XX	F, CHD	—	1 (>1)	4 (21)	60	79	90/98	MGN	HD->TX (11.0)	—	
NS 15[b]	46XX	F, CHD	—	1 (0.8)	1 (23)	3 (0.6)	40	46 L+R	MGN	HD-TX (8.7)	—	
NS16[c]	46XX	F	—	2d (10)	4d (18)	24 (1.0)	—	—	MC	— (2.3)	—	
NS17[d]	46XY	micropenis, cryptorchidism mental retardation, nystagmus	—	4 (13)	4 (8)	5 (1.0)	10	—	MC, IF	PD (4.3)	—	

Abbreviations are: DDS, Denys-Drash syndrome; F, normal external female genitalia; M, male genitalia; s. urog., sinus urogenitalis; CHD, congenital heart disease; Wilms' tumor: R, right kidney; L, left kidney; tri, mixed triphasic; s, stromal-predominant; Glomerulopathy: S_{Alb}, serum albumin; RF, renal failure; S_{Cr}; serum creatinine; ESRD, end-stage renal disease; NX, nephrectomy; RRT, renal replacement therapy; Histology: DMS, diffuse mesangial sclerosis; FSGS, focal segmental sclerosis; IF, interstitial fibrosis; MGN, mesangioproliferative glomerulonephritis; MC, minimal glomerular changes; PD, peritoneal dialysis; HD, hemodialysis; Tx, renal transplantation; AC, amnion cells.

Affected relatives: [a] sister had CNS/DMS/ESRD; [b] sisters; [c] sister had CNS/ESRD/MC; [d] cousin had CNS/DMS; [e] Mutations not previously described

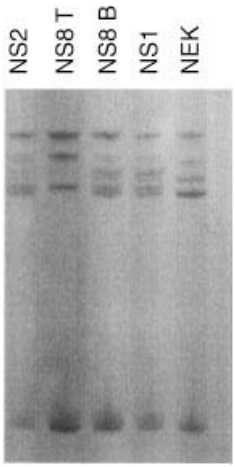

Fig. 1. Single-strand conformational polymorphism (SSCP) analysis of *WT1* exon 9 from blood (B) DNA of patients NS1, NS2 and NS8. Blood DNAs show heterozygous mobility pattern compared to the normal embryonal kidney (NEK) control. In the tumor (T) DNA from patient NS8 the wild-type allele is lost (only altered bands are seen).

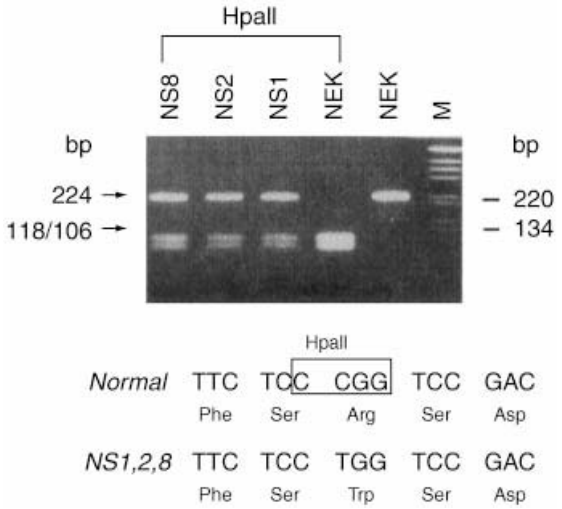

Fig. 2. HpaII digest of *WT1* exon 9 from blood DNA of patients NS1, NS2 and NS8 and a normal embryonic kidney (NEK) control. Loss of the recognition sequence by the mutation results in an undigested fragment of 224 bp; the digested products are 106 and 118 bp. The undigested NEK control DNA is shown for comparision. M: 1 kb size marker. Schematic drawing of the mutation with loss of *Hpa*II site and amino acid alteration.

changes amino acid 379 Gly close to the zinc coordinating histidine into a cysteine. Zinc binds with a higher affinity to cysteine than to histidine so that it is likely that both mutations change the structure of the protein and therefore prevent DNA binding.

Two infants with a female karyotype had an isolated form of infantile NS associated with DMS only (NS9, NS10). One of them presented the hot spot mutation and the other a missense mutation not previously described. The clinical course and pathoanatomical findings of these two infants were similiar to the DDS patients: both developed ESRD within a few weeks after discovery of nephropathy.

In the 10 patients with *WT1* mutations the age at apparent onset

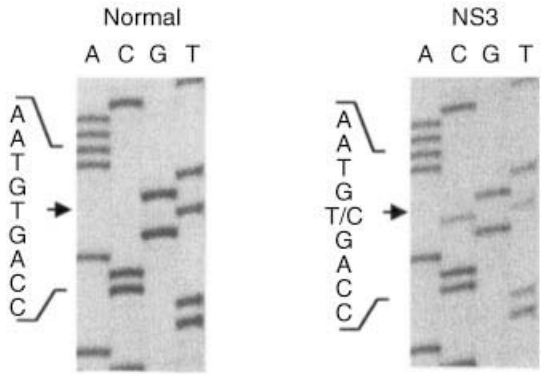

Fig. 3. Sequence analysis of *WT1* exon 9 from blood DNA of patient NS3 with the T>C alteration changing amino acid 385Cys to Arg.

of glomerulopathy ranged from the first days of life to 3.2 years. Renal histopathology in our patients with *WT1* mutations showed DMS in eight cases. A glomerulus from patient NS8 is shown in Figure 4. The shrunken tuft exhibits diffuse mesangial sclerosis with prominent matrix expansion. The podocytes on the tuft surface are inconspicuous. Two cases presented focal segmental glomerulosclerosis, one at the age of four days (NS5) and the other at 32 months (NS2) both combined with widespread interstitial fibrosis.

The WTs were detected at variable times in relation to the glomerulopathy and were always unilateral. In the seven genotypic males with *WT1* mutations genital findings were characterized either by severe hypospadias with cryptorchidism or apparently normal female external genitalia with an uterus and streak gonads (NS1–NS7).

Upon recognition of *WT1* mutations in two genotypic females with isolated infantile NS (NS9,10) we performed corresponding molecular studies in another seven NS patients not affected by WT. In none of the seven patients we found an anomaly of the *WT1* gene (Table 1). Five patients (NS11, NS14 to NS17) had a family history of progressive NS of early onset in a sibling or cousin. The sister of NS11, who was not analyzed for *WT1* mutations, died early in ESRD from the same disease. Five children had associated non-renal anomalies affecting the brain, eyes, heart, muscles and the skeletal system (NS11, NS12, NS14, NS15, NS17). The most severe malformation syndrome was found in NS12 with a very early onset of nephropathy and of ESRD. Two patients with isolated congenital or infantile NS in absence of *WT1* mutations presented with DMS (NS11 and NS12) the others (NS13 to NS17) had histopathologic findings compatible with mesangioproliferative glomerulonephritis or minimal glomerular lesions (Table 1).

The median age at onset of proteinuria was lower in patients without compared to those with *WT1* mutations (1 vs. 6 months), but the median time from onset to ESRD was shorter in the patients with mutations (2 vs. 19 months). However, due to the low number of patients compared from each group this difference was not statistically significant.

Furthermore, our investigation was supplemented by a group of six children with idiopathic NS with a late onset steroid resistant FSGS (between 2.5 and 13 years) that uniformly progressed to ESRD. None of these patients had a family history of NS or

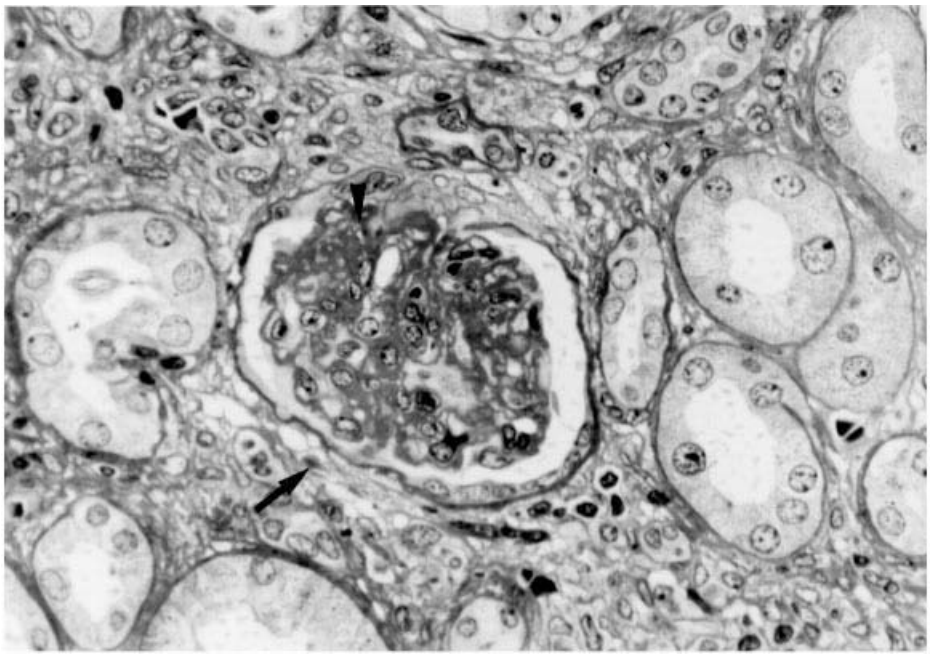

Fig. 4. Glomerulus from a patient with genetically verified Denys-Drash syndrome (DDS). Panels were photographed at ×170. The glomerulus is labeled with a arrow and the matrix expansion with a arrowhead.

associated anomalies. We failed to find any *WT1* mutations by screening exons 1 to 10 in these patients (data not shown).

No *WT1* mutations were detected in the parents of three and siblings of two patients with DDS or isolated congenital or infantile NS (see Table 1), demonstrating that these patients had new germline mutations.

DISCUSSION

Three different types of constitutional mutations have been described in DDS: missense, nonsense and splicing mutations of the *WT1* gene. Most are missense mutations that involve exon 9 corresponding to the third zinc finger (ZF) of the *WT1* gene; less frequently exons 6, 7 or 8 are affected. An actual review of the literature [11, 17–35] including our own patients demonstrates that 40% (24 of 60) of all cases exhibit the hot spot mutation R394W. Four mutations observed in our investigation were not described before. One of these changes the amino acid at the hot spot site directly involved in DNA binding to Gln instead of Trp. The other three most likely are structural changes of the protein ultimately also leading to lack of binding to the correct target sequence [38].

Our study confirms earlier reports that *WT1* mutations are present in children with the classical triad of DDS as well as in incomplete forms [11], that is, in absence of ambiguous genitalia or Wilms' tumor. All the mutations that we describe here are missense mutations. Similar to earlier studies we also found no relation between the type of missense mutation and the clinical phenotype of DDS. However, new genotype/phenotype correlations seem to emerge: (*1*) our own survey of a large number of Wilms' tumor patients with or without genital tract malformations, revealed that all, except one had nonsense mutations in the *WT1* gene and none of these patients had NS with ESRD ([36], see below); (*2*) recently a specific splicing mutation in the *WT1* gene was descibed in patients with Frasier syndrome, a rare disorder defined by male pseudohermaphroditism, progressive glomerulopathy and often associated with gonadoblastoma but not Wilms tumor [39]; (*3*) patients with *WT1* missense mutations always have NS followed by a rapid onset of ESRD associated with variable expressivity of a Wilms' tumor and depending on the primary sex with genital malformations. Therefore, the type of mutation in the *WT1* gene seems to dictate the accompanying disease, with the following order: a *missense* mutation causes a glomerulopathy with ESRD early in life and ambiguous genitalia in males and a high risk for Wilms tumor and possibly a low risk for gonadoblastomas; a *nonsense* mutation causes a high risk for Wilms tumor and less severe genital tract malformations in males; a *splicing* mutation causes glomerulopathy and pseudohermaphroditism in males and a moderate risk for gonadoblastoma, but no risk for Wilms tumor.

A Wilms' tumor was detected in only four of our ten patients presenting a *WT1* mutation, but it is notable that of the children without tumor two had died very early and three underwent bilateral nephrectomy before a WT was detected. One patient being 3.4 years old at the most recent observation with kidneys left *in situ* may still develop a WT. According to our own molecular analysis of 64 WT patients, *WT1* nonsense mutations are present preferentially in patients with stromal-predominant or triphasic Wilms' tumors and most of these are present in the germline [36]. Four Wilms' tumor patients of our series presented with hypospadias and/or cryptorchidism and one had slight proteinuria, but did not develop overt kidney disease and was therefore not counted as a DDS patient. It is of interest that the splice site mutation found in this child leads to a protein truncation in exon 7 at a similar site as in two other DDS patients, making it at least possible that these children may not have DDS [11]. In fact our own patient with the exon 6 mutation was initially described as a

possible case of DDS [40], but further analysis revealed that the patient did not have NS with ESRD [36] and it is now erroneously listed as a DDS patient in [11]. All Wilms' tumors from our DDS patients were also either triphasic or stromal-predominant, suggesting that germline mutations irrespective of the type (missense/nonsense) lead to the development of stromal-predominant or triphasic Wilms' tumors. However, for the development of a WT a second hit is needed that does not always occur, although the risk to develop a Wilms' tumor is over 90%, when a germline missense mutation is present [38].

Genital anomalies were observed in all our seven DDS patients with *WT1* mutations who had a male karyotype, ranging from severe forms of hypospadias, combined with a uterus and a vagina in two to external female genitalia, combined with streak (hypoplastic) gonads. We hesitate to define these genital anomalies generally as pseudohermaphroditism [41] as often reported in the literature, because a pathoanatomical examination of the gonads was not always performed in our series. The presence of germline *WT1* mutations in the ZF region is compatible with normal sexual development in the female but not in the male. It seems that the *WT1* gene is involved in the molecular events leading to the development of the male sex [12–14]. When the $WT1^{DDS}$ mutant protein (protein with an amino acid exchange) is present during the critical stage of sexual development it is thought to inhibit the activity of the normal protein, resulting in genital anomalies. The only exception is one apparently normal father with the hot spot mutation [29]. In this case the father may have a complementing mutation, abolishing the effect of the mutant protein, however, this has never been observed in any other case. Besides other possible explanations like genomic imprinting or mosaisism a mix up of the samples should also be considered and excluded by repeated sampling. In contrast, female DDS patients with the same spectrum of *WT1* mutations had a normal development of external genitalia, while in one infant internal genitalia were abnormal [17]. It is of interest that the male/female ratio of DDS patients reported with *WT1* mutations is increased to roughly 2:1. On the basis of our observation of *WT1* mutations in two females with isolated infantile NS we assume that the genotypic females are underrepresented among the DDS patients reported, because the normal phenotype leads less frequently to a molecular analysis of the *WT1* gene.

The glomerulopathy in DDS has been thoroughly described from a clinical and pathoanatomical point of view by Habib et al [6, 42, 43]. Our 10 patients with *WT1* mutations generally fit this picture. Severe proteinuria was observed between the newborn period and three years. In all patients renal failure was noted concomitantly with NS. The time between onset of proteinuria and ESRD was short (0–34 months). Interestingly, the longest time was observed in the three male patients with a Wilms' tumor (complete DDS), however, with only three patients in this category the significance of this observation needs confirmation by analyzing more patients. The pathoanatomical diagnosis was compatible with DMS in all children with *WT1* mutations except two infants with focal segmental glomerulosclerosis. FSGS has been described in a few other patients with DDS although no molecular genetic studies were described these patients [4]. In comparison in our patients without *WT1* mutations had an earlier onset of proteinuria (birth to 4 months), but the time from onset to ESRD tended to be shorter in the group with *WT1* mutations. At present it is difficult to decide if this difference in the rate of progression is characteristic for *WT1* associated nephropathy or is simply due to a different patient selection. The group without mutations was characterized by a high frequency of familial NS (5 of 7 patients) and of extrarenal/extragenital anomalies (5 of 7 patients) compared to the 10 patients with *WT1* mutations. We have not tested if the patients with familial NS without a *WT1* mutation were linked to the previously described SRN1 locus on chromosome 1 [44].

Based on our data and previous findings of *WT1* mutations in DDS patients, we propose that the presence of a *WT1* germline missense mutation and a nephrotic syndrome followed by rapid progression to ESRD should suffice to define DDS. We propose to abandon the division into complete and incomplete DDS and isolated NS in the future and classify all these patients with *WT1* missense mutations as DDS.

In conclusion, we suggest that patients with progressive forms of early onset NS, even in absence of renal biopsy, should undergo analysis of the *WT1* gene independent on the finding of a WT or genital abnormalities. The presence of DMS makes this research mandatory. In males, ambiguous genitalia are an important indicator of *WT1* mutations. In females progressive glomerular disease alone may be revealing. In about half of patients with DDS no kidney tumor is recognized when NS becomes manifest. *WT1* mutations may be found in children presenting with NS or renal failure after one year of age and in infants without DMS; however, investigators are invited to provide more detailed morphological description of renal histology from patients with *WT1* mutations in the future. The finding of a missense mutation in the *WT1* gene indicates an increased risk to develop Wilms' tumor and possibly gonadoblastomas in later life, even after start of renal replacement therapy. Therefore, careful monitoring of all patients with *WT1* mutations is required. Regular renal imaging may be necessary to evaluate the need for bilateral nephrectomy in patients with *WT1* missense mutations who have attained ESRD.

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