

# Genotype/Phenotype Correlation in Nephrotic Syndrome Caused by *WT1* Mutations

Gil Chernin,\* Virginia Vega-Warner,\* Dominik S. Schoeb,\* Saskia F. Heeringa,\*  
Bugsu Ovunc,\* Pawaree Saisawat,\* Roxana Cleper,<sup>†</sup> Fatih Ozaltin,<sup>‡</sup>  
Friedhelm Hildebrandt,<sup>\*§</sup> and Members of the GPN Study Group

\*Departments of Pediatrics and of Human Genetics, University of Michigan, Ann-Arbor, Michigan; <sup>†</sup>Pediatric Nephrology Unit, Schneider Children's Medical Center, Petah Tiqva, Israel; <sup>‡</sup>Department of Pediatrics, Hacettepe University, Ankara, Turkey; and <sup>§</sup>Howard Hughes Medical Institute, Chevy Chase, Maryland

**Background and objectives:** The risk of developing Wilms tumor (WT) can be present or absent in patients with nephrotic syndrome (NS) caused by *WT1* mutations. Here, the genotype/phenotype correlation regarding the outcome and risk for WT in 52 patients from 51 families with NS due to *WT1* mutations is described.

**Design, setting, participants, & measurements:** This study followed 19 patients with mutations in intron 9 splice donor site (KTS mutations), 27 patients with missense mutations, 4 patients with nonsense mutations, 1 patient with a splice site mutation in intron 8, and 1 patient with a deletion.

**Results:** Twenty-four different *WT1* mutations were detected. Sixteen of the 19 patients with KTS mutations were females. These patients had isolated NS if karyotype was 46,XX and Frasier syndrome if karyotype was 46,XY. Patients with KTS mutations presented at a significantly older age and with a slower progression toward chronic kidney disease (CKD) stage 5, compared with missense mutations. Patients with nonsense mutations presented initially with WT. Six patients with missense mutations developed WT after the diagnosis of NS (interval-range from NS onset to WT of 0.1 to 1.4 years).

**Conclusions:** (1) KTS mutations cause isolated NS with absence of WT in 46,XX females. (2) KTS mutations cause Frasier syndrome with gonadoblastoma risk in 46,XY phenotypic females. (3) KTS mutations cause NS with a slower progression when compared with missense mutations. (4) Missense mutations can occur with and without WT. (5) *WT1* analysis is important in young patients with NS for early detection and tumor prophylaxis.

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**W***T1* mutations can lead to three distinct clinical entities that are associated with the glomerular disease of steroid-resistant nephrotic syndrome (SRNS): (1) Denys-Drash syndrome (DDS) (1); (2) Frasier syndrome (FS) (2); and (3) isolated NS (3,4). DDS is classically characterized by the triad of infantile SRNS, ambiguous genitalia, and Wilms tumor (WT) (5,6). The characteristic renal biopsy finding in DDS is diffuse mesangial sclerosis (DMS) (6,7). Incomplete forms of DDS have been reported with early glomerulopathy associated with either major urogenital abnormalities or WT (6). FS is characterized by the association of SRNS with male pseudohermaphroditism. In FS, the onset of NS occurs later than in DDS with typical histologic findings of focal segmental glomerulosclerosis (FSGS) (6). The term “isolated NS” is used here to denote NS without accompanying WT or major urogenital abnormalities. In more than 95% of cases with NS and *WT1* mutations, the mutations are located in exon 8 or 9 of *WT1* (4,8–10). FS is caused by mutations in the

donor splice-site of intron 9. The predicted consequence of mutations in this site is the heterozygous loss of the three amino acid residues KTS (referred from here as KTS mutations) with a resulting reduction in the ratio of the isoforms +KTS/-KTS (11).

There is still uncertainty about the appropriate management of these patients. An important clinical issue emerges in cases where the diagnosis of SRNS caused by *WT1* mutation is made, yet without the diagnosis of a tumor. The known risk of WT in DDS patients and of gonadoblastoma in FS patients creates a challenge to the clinician in terms of surveillance and tumor prevention (12,13). This uncertainty led us to investigate the clinical outcome in a large cohort of 52 patients from 51 different families with *WT1* mutations as the cause of NS. Our goal was to describe the genotype/phenotype correlation in this large group of patients. Our hypothesis was that specific mutations may predict isolated NS without the manifestation of WT or gonadoblastoma.

## Materials and Methods

### Patients and Data Recruitment

Patient recruitment following informed consent and the clinical evaluation has been described previously (14). Upon termination of this study (July 2009), a follow-up questionnaire was sent to the treating physicians with questions regarding tumor occurrence, novel extrarenal manifestations, karyotype if not done before, the development of

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**Correspondence:** Dr. Friedhelm Hildebrandt, University of Michigan Health System, 8220C MSRB III, 1150 West Medical Center Drive, Ann Arbor, MI 48109-5646. Phone: +1-734-615-7285; Fax: +1-734-615-1386, -77; E-mail: fhilde@umich.edu

chronic kidney disease (CKD) stage 5 since recruitment, and kidney transplantation success, if done. Fifty-two patients from 51 families were found to have NS with a heterozygous mutation in the *WT1* gene (Supplemental Table 1). Eighteen patients were briefly described in previous mutation analysis of SRNS patients (3,4,15,16), and one patient (A1655) was described in a case report (17) (Supplemental Table 1). Standard steroid treatment and responses to steroid therapy were defined according to previously published guidelines (18,19).

### Diagnosis of *WT1* Mutation by Direct Sequencing

Mutation analysis was performed by exon-flanking PCR with consecutive direct sequencing of exons 8 and 9 of *WT1* as described previously (3,4). *WT1* analysis was limited to exons 8 and 9 as the NS-causing mutations of this gene were described in these two exons in more than 95% of the patients (4). The diagnosis of a *de novo* mutation or segregation from a parent was established when DNA samples of the parents were available. All mutations considered disease causing were absent from 90 healthy control individuals.

### Categories of *WT1* Mutations for Genotype/Phenotype Correlation

Patients were classified for genotype/phenotype correlation of *WT1* mutations according to the type of mutation found. Patients with the KTS mutations in intron 9, IVS9 + 4 C>T or IVS9 + 5 G>A, were classified as one group on the basis of previous data suggesting that these mutations both predominantly cause FS or isolated NS (3,6). All patients with a missense mutation were considered as another group under the suggestion that these mutations mainly cause early onset DDS or isolated DMS (3,20).

### Clinical Analysis and Exclusion Criteria for Genotype/Phenotype Correlation

DDS was defined by the minimal diagnostic criteria of disease presentation before 2 yr of age with glomerulopathy and one of the following: (1) urogenital abnormalities; (2) WT (6). The complete form

of FS was defined as male pseudohermaphroditism (female phenotype with karyotype 46,XY) and progressive glomerulopathy after the age of 2 years (6). An incomplete form of FS was defined in 46,XY phenotypic male patients with progressive glomerulopathy after the age of 2 years and major urogenital abnormalities (21). Isolated NS was defined by the lack of the syndromic features of DDS or FS.

For genotype/phenotype correlation of age of NS onset, we included all patients with KTS mutations or missense mutations for whom we could obtain the age of onset. We excluded patients with KTS mutations or missense mutations from the evaluation of interval between NS onset and CKD stage 5 (see Figure 1) for one or more of the following reasons: (1) patients without an exact date given of disease onset and/or CKD stage 5 diagnosis; (2) patients who reached CKD stage 5 due to bilateral nephrectomy, which was performed either as a treatment of WT or as a preventive measure.

Exclusion criteria from genotype/phenotype correlation (see Table 3) of WT, FS, and isolated NS were: (1) patients who died before the age of 5 years without a fully developed syndrome (DDS with WT or FS); (2) patients without WT who had a preventive bilateral nephrectomy before the age of 5 years or within less than 2 years after NS onset.

### Statistical Analysis

For comparison of NS age of onset between groups with different type of mutations, we fit a *t* test comparing the log (age) of each group. For comparison of interval from NS onset to CKD stage 5 between groups with different types of mutations, we used the Log-Rank test. For each comparison,  $P < 0.05$  was considered as statistically significant.

## Results

### Mutation Analysis of 52 Patients from 51 Families with NS and *WT1* Mutations

Mutation analysis and clinical data of all 52 patients with NS and *WT1* mutation are presented in Supplemental Table 1. In

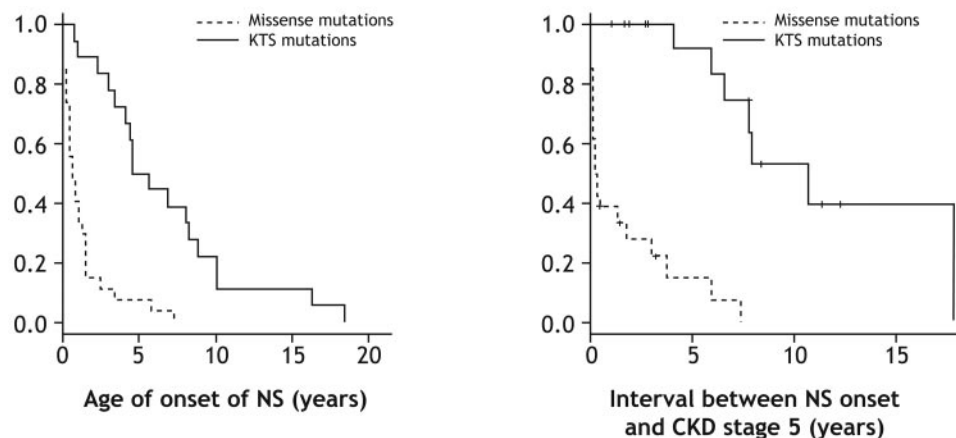


Figure 1. (Left) NS manifests later in patients with KTS mutations compared with patients with missense mutations in exons 8 or 9 of *WT1*. Kaplan-Meier survival analysis of interval from birth to onset of NS for 18 patients with splice site mutation in intron 9 (KTS mutations) of *WT1* and 27 patients with missense mutations in *WT1*. The age of NS onset was significantly older in the KTS mutation group compared with patients with missense mutations (*t* test,  $P < 0.006$ ). (Right) KTS mutations lead to a slower progression of NS toward CKD stage 5 than missense mutations. Kaplan-Meier survival analysis of interval between onset of NS and CKD stage 5 in 17 patients with splice site mutation in intron 9 (KTS mutations) compared with 26 patients with missense mutations. The vertical latches represent the interval between NS onset and termination of this study for each patient that did not reach CKD stage 5. The interval between onset of NS and CKD stage 5 was significantly longer in the KTS mutation group compared with patients with missense mutations (Log-Rank test,  $P < 0.006$ ).

the 51 families, we found 18 families (and 19 patients) with KTS mutations, 27 families with missense mutations, four families with nonsense (truncating) mutations, one family with mutation in the donor splice-site of intron 8, and one family with an in-frame deletion. Overall, 16 patients had the minimal diagnostic criteria of DDS. Five patients had the complete form of DDS (*i.e.*, with a major urogenital malformation and WT). Another 11 patients had the incomplete form of DDS, of which seven patients were found to have only WT and four patients had only a major urogenital malformation without WT.

We detected 24 different mutations in 51 families (Supplemental Table 1). There were two different KTS mutations, 19 missense mutations, one nonsense mutations, one mutation in the donor splice-site of intron 8, and one in-frame deletion. Six of the 24 different mutations in this cohort (25%) are novel (Table 1). The frequency of mutations was uneven by distribution, as the three most common mutations were found in 24 of the 51 families (47%). These were the IVS9 + 5 G>A in nine families, IVS9 + 4 C>T in eight families, and p.R394W in seven families. Segregation of mutations was found only in one family, A3110, with affected mother and daughter (A3110 I-2 and A3110 II-1, respectively). All other mutations examined were mutations that occurred *de novo* in the affected children.

#### Genotype/Phenotype Correlation for Age of NS Onset in Patients with KTS or Missense Mutations

For genotype/phenotype correlation of age of NS onset, we included 45 of the 46 patients with KTS or missense mutations for whom we could obtain the age of onset. That led to the exclusion of one patient with a KTS mutation. Eighteen patients had KTS mutations, and 27 patients had missense mutations. Kaplan-Meier survival analysis was performed for age of NS onset (Figure 1, left). Patients with KTS mutations in intron 9 had a statistically significant older age of NS onset (median age 5.1 years, range 0.8 to 18.5 years;  $n = 18$ ) compared with patients with missense mutations (median age 0.8 years, range 0 to 7.3 years;  $n = 27$ ) with  $P < 0.006$  ( $t$  test). We conclude that KTS mutations lead to onset of NS, which is delayed by a median of 4.3 yr compared with missense mutations of *WT1* (Figure 1, left).

#### Genotype/Phenotype Correlation for Interval Between NS Onset and CKD Stage 5 in Patients with KTS or Missense Mutations

Three patients (two with KTS mutations and one with a missense mutation) were excluded from the evaluation of interval between NS onset and CKD stage 5. That led us to evaluate 43 of the 46 patients with KTS or missense mutations. Seventeen of these patients had KTS mutations, and 26 patients had missense mutations. Kaplan-Meier survival analysis was performed for interval between NS onset and CKD stage 5 (Figure 1, right). Ten patients with KTS mutations and four patients with missense mutations did not reach CKD stage 5 upon termination of study (see Figure 1, right, and Supplemental Table 1). Patients with the KTS mutations had a statistically significant longer interval from NS onset to CKD stage 5 (median interval of 8 years) when compared with patients with missense mutations (median interval of 0.2 years) with  $P < 0.006$  (Log-Rank test). We conclude that KTS mutations delay the interval between onset of NS and CKD stage 5 when compared with missense mutations.

#### NS and WT in Patients with *WT1* Mutations

Twelve patients in the cohort had NS with WT (Table 2). The age of WT diagnosis ranged from 0.3 to 1.7 years. In six patients, the diagnosis of WT was made either before or concomitantly with the diagnosis of NS. In these six patients, the interval from WT diagnosis to NS diagnosis was very inhomogeneous, varying from 0 to 13 years. Four of these six patients had the nonsense mutation p.R390X.

The other six patients of the 12 with WT had WT diagnosed after NS onset. In these six patients, WT occurred at 0.6 to 1.6 years of age. The interval range from onset of NS to WT was 0.1 to 1.4 years. In contrast to the six patients with WT diagnosed before NS, who had mostly nonsense mutations, all six patients diagnosed with WT after NS carried missense mutations (Table 2).

#### Genotype/Phenotype Correlation Regarding WT, FS, and Isolated NS in 40 NS Patients with *WT1* Mutations

Clinical data to examine genotype/phenotype correlation regarding WT, FS, or isolated NS was available in 40 of the 52

Table 1. Six novel *WT1* gene mutations in patients with nephrotic syndrome

Patient	Mutation, <sup>a</sup> Consequence	Age of NS Onset (years)	Clinical Course	Renal Biopsy
A1114	c.1133C>G, p.T378R	1.3	DDS	DMS
A1325	c.1180C>G, p.R394G	0.5	NS with WT, early death	DMS
A1438	c.1193T>G, p.L398R	7.3	Isolated NS	FSGS
A1696	c.1123_1128delAGGAGA, p.R375_R376del	0.1	NS, nephrectomy at age 1.1 years	Not done
A1899	c.1078 T>C, p.C360R	0.4	DDS; death of respiratory failure	DMS
A2925	IVS 8 + 1 G>A, splice defect	0.1	NS; death of infection	Not done

DDS, Denys-Drash syndrome; DMS, diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis.

<sup>a</sup>All mutations are heterozygous.

Table 2. Clinical features of 12 NS patients with Wilms tumor and *WT1* mutation

Patient	Gender	Mutation	Age at NS Onset (years)	Age at WT Diagnosis (years)	Kidney Biopsy
Patients with manifestation of WT before or concomitantly with NS diagnosis					
A133	f	p.H401R	0.3	0.3	No data
A822	m	p.R390X	1.0	1.0	MCNS
A1251	m	p.R390X	1.7	1.7	No data
A1655	f	p.R390X	3.9	1.0	MPGN1
A1998	m	p.R390X	4.0	1.0	FSGS
A2092	f	p.D396N	1.5	1.5	No data
Patients with manifestation of NS before WT diagnosis					
F1031	f	p.C388R	0.1	1.5	DMS
A1114	m	p.T378R	1.3	1.4	DMS
A1325	f	p.R394G	0.5	1.2	DMS
A2329	f	p.R394W	0.6	1.5	FSGS
A2407	m	p.R394W	1.5	1.6	Global sclerosis
A3021	m	p.R394L	0.2	0.7	no data

f, female; m, male; MCNS, minimal change nephrotic syndrome; MPGN1, membranoproliferative glomerulonephritis type 1.

patients of the cohort (Table 3). Nine patients were excluded from the correlation due to early preventive bilateral nephrectomy (<2 years after disease onset) precluding assessment of WT onset. Another three patients were excluded due to early death (<2 years after disease onset) precluding assessment of WT onset. Of the 18 patients with KTS mutations, 16 were phenotypically females and had either FS ( $n = 4$ ) or isolated NS ( $n = 12$ ) (Table 3, first two columns). All four phenotypic females with karyotype 46,XY manifested with FS and streak gonads, while all 12 females with karyotype 46,XX were found to have isolated NS (Table 3). Two male patients, F963 and F1073, both with the KTS mutation IVS9 + 5 G>A, were found to have karyotype 46,XY and FSGS associated with hypospadias and testicular atrophy. These two patients were considered as a variation of FS and are grouped together with FS in Table 3, second column (21). Three of the four phenotypic female patients with FS developed gonadoblastoma.

All four patients with a nonsense mutation had the same mutation p.R390X (Table 3, fifth column). All four patients presented initially with WT with onset of NS either concomitantly or after the diagnosis of WT.

In 18 of the 27 patients with a missense mutation, clinical data were sufficient to assess genotype/phenotype correlation (Table 3, columns 3 and 4). Seven of the 18 patients had the p.R394W missense mutation. The other 11 patients had 11 different missense mutations. Patients with missense mutations presented either with isolated NS ( $n = 10$ ), with NS and WT only (incomplete form of DDS;  $n = 6$ , see Table 3, columns 3 and 4), or with the complete form of DDS (urogenital abnormalities and WT;  $n = 2$ , see Table 3, columns 3 and 4). Eight of the 10 patients with isolated NS and a missense mutation had NS onset in the first 2 yr of life (range 0 to 1.5 years; see Figure 1, left).

## Discussion

In this study we describe the long-term follow-up and genotype/phenotype correlation in a large cohort of 52 patients from 51 different families with NS and a mutation in exons 8 and 9 of *WT1*. To the best of our knowledge, this is so far the largest published cohort of such kind. This study analyzed *WT1* mutations only in exons 8 and 9, as mutations in these exons accounts for more than 95% of SRNS cases caused by *WT1* mutations (4). Importantly, the relationships describe here are limited to patients with NS and may not be generalized to patients with WT, who have yet to develop NS or patients with *WT1* mutation who have not developed NS.

Our main findings on genotype/phenotype correlation may give guidelines regarding the pathologic significance of certain *WT1* mutations in NS patients in the following way: (1) KTS mutations are associated with a later onset of NS and slower progression of NS toward CKD stage 5, when compared with missense mutations (Figure 1). These results are compatible with previous data (3,4,20). (2) None of the NS patients with KTS mutations developed WT. One previous report described a patient with a KTS mutation and FS associated with WT, but our data suggest that this association is an exception (22). (3) In NS patients with KTS mutations: If phenotypic female gender and karyotype were in concordance, patients developed isolated NS only. (4) In phenotypic female patients with NS and KTS mutations: If karyotype was 46,XY, patients developed FS with risk of gonadoblastoma. These findings are in agreement with previous literature (4,6). Two phenotypically NS male patients with the mutation IVS9 + 5 G>A and karyotype 46,XY were found to have FSGS associated with hypospadias or testicular atrophy. It was previously reported of two 46,XY male patients with NS and the IVS9 + 4 C>T mutation associated

Table 3. Genotype/phenotype correlation regarding WT, FS, and isolated NS in 40 patients with NS and WT1 mutations in exons/introns 8 or 9

Syndrome	+KTS/-KTS Splice Mutations			Missense Mutations		Nonsense Mutations		Number of Patients
	IVS9 + 4 C>T, Indiv/KT-Gender/Gonads	IVS9 + 5 G>A, Indiv/KT-Gender/Gonads		R394W, Indiv/KT-Gender/Gonads	Other missense, Indiv/KT-Gender/Gonads	R390X, Indiv/KT-Gender/Gonads		
Absence of WT	2	4		0	0	0		6
	F1194/M-F/GB	F963/M-M/testicular atrophy						
	A3110 II-1/M-F/GB	F1073/M-M/hypospadias						
		A1688/M-F/GB						
		A2520/M-F/SG						
Isolated NS <sup>a</sup>	7	5		5	5	0		22
	F953/F-F/nl	F921/F-F/nl		A2203/F-F/nl	F734/F-F/nl			
	F999/F-F/nl	F1280/F-F/nl		A2207/M-M/nl	A644/F-F/nl			
	A670/F-F/nl	A562/F-F/nl		A2353/F-F/nl	A1276/F-F/nl			
	A1463/F-F/nl	A2074/F-F/nl		A3028/F-F/nl	A1277/F-F/nl			
	A1850/?-F/nl	A2328/F-F/nl		A3038/F-F/nl	A1438/?-M/nl			
	A1853/F-F/nl							
	A3110 I-2/F-F/nl							
Presence of WT								
Complete or incomplete DS	0	0		2	6	4		12
				A2329/F-F/nl	F1031/F-F/nl	A822/M-M/AG		
				A2407/M-M/nl	A133/F-F/nl	A1251/M-M/AG		
					A1114/M-M/AG	A1655/F-F/nl		
					A1325/F-F/nl	A1998/M-M/AG		
					A2092/F-F/nl			
					A3021/M-M/AG			
Total	9	9		7	11	4		40 <sup>b,c</sup>

AG, ambiguous genitalia; KT, karyotype; nl, normal; NS, nephrotic syndrome; SG, streak gonads; WT, Wilms' tumor; ?, Karyotype not done.

<sup>a</sup>Isolated NS defined as NS without DDS, FS, or WT. Atrial septal defect, laryngomalacia, left ventricular hypertrophy, aberrant renal artery, and cleft palate were not considered as part of DDS or FS.

<sup>b</sup>Three patients in the total cohort of 52 died prematurely (<2 years from NS onset) without the diagnosis of Wilms tumor and were excluded from this genotype/phenotype correlation.

<sup>c</sup>Nine patients in the total cohort of 52 were excluded from this genotype/phenotype correlation due to prophylactic nephrectomy early in the course of the disease (<2 years of NS onset).

with either hypospadias (23) or normal phenotype (24). Our data suggest that these reports are indeed the exception to the rule in patients with the KTS mutations. Patient A1271 with the mutation IVS9 + 5 G>A had DMS with chordee and right hydrocele of testis but was excluded from the definite clinical classification due to an early preventive bilateral nephrectomy. Although similar DDS phenotype was previously reported in patients with KTS mutations, our data suggest that this is an atypical presentation of KTS mutations (25). (5) All four NS patients with the nonsense mutation (p.R390X) developed WT. Other nonsense mutations of *WT1* were previously reported with a variety of phenotypes, almost always with WT occurrence (26,27). (6) NS patients with missense mutations presented in this cohort either in the form of DDS with WT or in the form of isolated NS. Therefore, missense mutations do not allow any conclusion regarding presence or absence of WT risk. None of the NS patients with missense mutations had FS, suggesting that the previous description of a missense mutation in a FS patient is anecdotal (21).

Interestingly, all 12 patients with NS and WT developed the tumor early in life, before the age of 1.7 years. This observation is in agreement with previous findings of an earlier age of tumor detection in patients with WT and *WT1* mutations when compared with patients with WT without a *WT1* mutation (28).

This study, as previous studies, did not reveal a specific missense mutation that would be associated with a reduced risk to WT occurrence (20,29). Under these circumstances, a preventive bilateral nephrectomy to individuals with CKD stage 5 and missense mutations of *WT1* may be warranted. The lack of association in this study may have been influenced by the exclusion from clinical classification of nine patients with missense mutations due to early preventive nephrectomy or death, and by the fact that all missense mutations, other than p.R394W, were found each in only one patient.

Once a *WT1* mutation is found in a patient with NS, this raises a concern of increased risk to develop WT or gonadoblastoma (6,12). The clinician and the patient then need to make a decision regarding the options of surveillance and possible prevention of a tumor. Potential benefits of periodic screening for WT diagnosis are early detection and treatment of WT, although there is no definite evidence that this strategy will decrease overall mortality or tumor stage (30). Potential drawbacks are anxiety for the patient and his/her family and unnecessary tests or procedures (31). In many centers, a bilateral preventive nephrectomy is performed in cases of NS associated with *WT1* mutation, especially when the patient has reached CKD stage 5 (12). Indeed in our cohort, nine patients had a preventive bilateral nephrectomy. The initial suggestion for a preventive nephrectomy in patients with DDS was published in the pre-era of genetic screening for *WT1* mutations (32). The drawbacks of a preventive nephrectomy are the additive risk of a bilateral nephrectomy procedure and the potential loss of residual renal function in CKD stage 5 patients (33,34). In light of our findings, we suggest a flow chart, for the risk evaluation and surveillance of patients with NS and *WT1* mutations, that can provide guidance when counseling parents of children with NS and *WT1* mutations (Figure 2).

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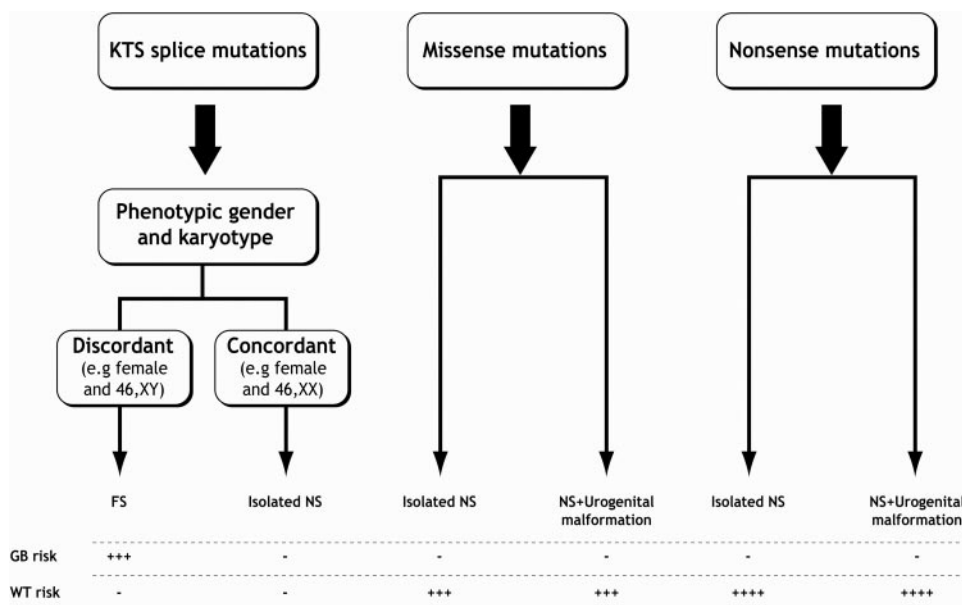


Figure 2. Tumor risk assessment in patients with nephrotic syndrome and *WT1* mutations in exons 8 or 9. GB, gonadoblastoma.

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## Disclosures

None.

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