Mother-to-Child Transmitted \textit{WT1} Splice-Site Mutation Is Responsible for Distinct Glomerular Diseases

ERICK DENAMUR,* NATHALIE BOCQUET,* BEATRICE MOUGENOT,‡ FRANCIS DA SILVA,* LAURENCE MARTINAT,† CHANTAL LOIRAT,† JACQUES ELION,* ALBERT BENSMAN,‖ and PIERRE M. RONCO§
*Laboratoire de Biochimie Génétique and Institut National de la Santé et de la Recherche Médicale U458, †Service de Néphrologie Pédiatrique, Hôpital Robert Debré; ‡Département de Pathologie and †Service de Néphrologie, Hôpital Armand-Trousseau, Assistance Publique-Hôpitaux de Paris, Paris, France.

Abstract. Mutations in the Wilms’ tumor suppressor gene (\textit{WT1}) are linked with Denys-Drash syndrome (DDS), a rare childhood disease characterized by diffuse mesangial sclerosis and renal failure of early onset, XY pseudohermaphroditism, and high risk of Wilms’ tumor. KTS (lysine-threonine-serine) splice site mutations in \textit{WT1} intron 9 have been described in patients with Frasier syndrome, another rare syndrome defined by focal and segmental glomerulosclerosis (FSGS), XY pseudohermaphroditism, and frequent occurrence of gonadoblastoma. Cases of Frasier syndrome raise the question whether splice site mutations may also be found in XX females with isolated FSGS. A girl (index case) presented with the nephrotic syndrome at 9 mo of age. The diagnosis of DDS was based on the finding of diffuse mesangial sclerosis in the kidney biopsy and of a XY karyotype. The index case’s mother had had proteinuria since she was 6 years of age. A renal biopsy was performed when she was 28 and disclosed FSGS. The same splice site mutation in intron 9 (\textit{WT1} 1228+5 G→A) involving one allele was found in the child and in her mother, but not in other members of the kindred (including the parents, the two brothers, and the two sisters of the index case’s mother) who were free of renal symptoms. Quantification of \textit{WT1} +KTS/−KTS isoforms in the index case’s father and one index case’s maternal uncle showed a normal +KTS/−KTS ratio of 1.50. In contrast, the index case and her mother had a low ratio (0.40 and 0.34, respectively), within the range reported in Frasier syndrome. In conclusion, this study shows that the KTS splice site mutation is not specific for Frasier syndrome, but that it can also be found in DDS and in a normal female (XX) with FSGS, a woman who achieved normal pregnancy. It is suggested that \textit{WT1} splice site mutations should be sought in phenotypically normal females who present with FSGS or with related glomerulopathies of early onset.

Primary (idiopathic) focal and segmental glomerular sclerosis (FSGS) is a frequent glomerular disease. It is identified in 15 to 20% of adults and 7 to 15% of children presenting with idiopathic nephrotic syndrome and often leads to end-stage renal failure (1). It is currently believed that primary FSGS is caused by alterations of the glomerular visceral epithelial cells (also referred to as podocytes) induced by a circulating permeability factor (2) or by an intrinsic podocyte cellular defect. The role of genetic and ethnic factors has been suspected on the basis of cases occurring in multiple families and sibling pairs (3–6) and of the much greater incidence of the disease in Africans (7). A locus for inherited FSGS was recently shown to map to chromosome 19q13 (8).

Frasier syndrome is a rare disease defined by XY pseudoher-
erentially with transcription factors, whereas +KTS isoforms associate with splicing factors (16). In Frasier syndrome, the +KTS/−KTS isoform ratio is diminished due to the under-representation of the +KTS isoform (12–14).

The renal phenotype is milder in Frasier syndrome than in Denys-Drash syndrome (DDS), which is characterized by diffuse mesangial sclerosis that normally develops within the first year of life and leads to end-stage renal failure, by XY pseudohermaphroditism, and by a high risk of developing Wilms’ tumor (17). DDS is usually linked with de novo heterozygous WT1 missense mutations (18). It has recently been shown that a heterozygous zinc finger truncation of murine WT1 results in the characteristic urogenital abnormalities of DDS (19).

We report on a family in which the WT1 1228+5 G→A mutation was transmitted from a mother to her only child who had DDS. The mother suffered from nephrotic syndrome caused by FSGS. Her renal function was normal. We studied the relative ratio of +KTS/−KTS transcripts in this family in an attempt to unravel the molecular basis of the phenotypic polymorphism.

Materials and Methods

Patients

The 30-yr-old mother is Portuguese (Table 1). Proteinuria was detected at the age of 6. Twenty-four-hour urinary albumin excretion was stable, in the order of 2 to 3 g, until the patient became pregnant at the age of 27. Eclampsia occurred at 29 wk of gestation on April 16, 1996. Serum creatinine was 0.9 mg/dl, serum albumin 2 g/dl, and uric acid 6.4 mg/dl. The urinary sediment, the coagulation, and the liver tests were normal. A cesarean section was performed on the same day.

The patient was referred to us 1 yr later for persistent nephrotic syndrome. BP was 130/70 mmHg. Clinical examination was normal. Laboratory tests showed: hemoglobin, 13.2 g/dl; total serum proteins, 5.4 g/dl; serum albumin, 2.3 g/dl; serum creatinine, 0.9 mg/dl. Twenty-four-hour proteinuria was 4 g. The urinary sediment showed 10,000 red blood cells/ml. Intravenous pyelography did not reveal renal malformation or vesico-ureteral reflux. A renal biopsy disclosed typical lesions of FSGS in 30% of the glomeruli (Figure 2A), 20% obsolescent glomeruli, significant tubulointerstitial lesions with numerous foam cells, and chronic vascular changes. Electron microscopy showed nonspecific lesions that consisted of mesangial matrix expansion, irregular enlargement of the subendothelial aspect of the glomerular basement membrane, segmental effacement of foot processes, and frequent capillary wall wrinkling. Persistent pelvic pain prompted ultrasound examination, which disclosed a homogeneous uterine mass with reduced echogenicity that was shown at surgery to be an endometriotic nodule. The ovaries had a normal macroscopic appearance. Biopsies showed follicles at all stages of maturation. Gonadotropic axis tests were within the normal range.

The child (index case) was referred to us at the age of 9 mo for investigation of nephrotic syndrome (Table 1). Clinical examination showed normal female external genitalia and edema of the lower limbs and ascites. Urinary albumin excretion was 2.2 g/dl. Blood laboratory tests showed: serum creatinine, 0.3 mg/dl; total proteins, 2.9 g/dl; serum albumin, 0.7 g/dl. The urinary sediment contained 600,000 red blood cells/ml. Ultrasound examination disclosed two normal-sized kidneys, a small uterus, but gonads could not be visualized. A renal biopsy was performed at the age of 9 mo. Light microscopy (Figure 2, B and C) showed typical diffuse mesangial sclerosis. All glomeruli were modified by prominent matrix expansion with hypertrophy and vacuolation of some podocytes. Some capillary tufts were shrunken with diminished patency of the capillary lumens and occasional basement membrane thickening. The interstitium was

Table 1. Characteristics of the two patients carrying WT1 intron 9 donor splice site heterozygous mutationa

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Age at First Symptoms (yr/mo)</th>
<th>Histology</th>
<th>Serum Creatinine at Last Follow-Up (mg/dl)</th>
<th>Karyotype</th>
<th>External Genitalia</th>
<th>Gonadoblastoma</th>
<th>Age at Follow-Up without WT (yr/mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>1228+5 G→A De novo</td>
<td>6/? FSGS</td>
<td>1.2</td>
<td>46,XX Female No</td>
<td>30/9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daughter</td>
<td>1228+5 G→A Inherited</td>
<td>0/9 DMS</td>
<td>0.6</td>
<td>46,XY Female No</td>
<td>2/10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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* WT, Wilms’ tumor; FSGS, focal segmental glomerulosclerosis; DMS, diffuse mesangial sclerosis.
slightly fibrotic and contained atrophic tubules. Electron microscopy confirmed the increase in mesangial matrix and the enlargement and vacuolation of the podocytes, with irregular effacement of foot processes. Karyotype analysis showed 46 chromosomes including XY. On last examination in January 1999 at the age of 2 yr and 9 mo, the child’s body weight was 12.5 kg and her height was 90 cm. BP was controlled by beta blockers and calcium channel antagonists. She had edema of the legs and moderate ascites. Laboratory tests showed: hemoglobin, 16.2 g/dl; serum creatinine, 0.6 mg/dl; total serum proteins, 3.7 g/dl; serum albumin, 1.8 g/dl. Abdominal ultrasonography did not reveal Wilms’ tumor. Luteinizing hormone-releasing hormone test was within normal range.

We failed to detect albuminuria and urinary sediment abnormalities in the parents and the four siblings of the index case’s mother, as well as in the index case’s father. There was no history of consanguinity.

**Detection of WT1 Mutations**

WT1 mutations were identified by direct sequencing of a WT1 PCR product obtained from white blood cell genomic DNA as described previously (20).

**Quantification of WT1 +KTS/−KTS Isoforms**

Both +KTS and −KTS WT1 alternative transcripts (113 and 104 bp, respectively) were amplified by semi quantitative reverse transcription-PCR from total RNA extracted from patients’ lymphoblastoid cell lines (index case and relatives, except index case’s mother) or from renal and ovarian biopsies (index case’s mother). We used the same method as described previously (12) except that the WTRT1 primer was 5′ fluorescein-labeled and that no radioactivity was used. PCR products were resolved by capillary electrophoresis on an ABI Prism 310 (Perkin Elmer). The isoform ratio was calculated as the ratio of the area under the curve of the two peaks separated by 9 bp. The given ratio values are the means of at least three experiments.

**Results and Discussion**

The WT1 1228+5 G→A mutation was found in one allele in the mother and her daughter (index case). There was no evidence for mosaicism, as the peaks corresponding in the sequence reaction to the normal and mutated nucleotides at position 1228 were of the same height. No additional mutations were observed in the WT1 exons 8 and 9 of the index case. The 1228+5 G→A mutation was not detected in the index case’s father or in the index case’s maternal grandparents or the four siblings of the index case’s mother.

Quantification of WT1 +KTS/−KTS isoforms by semi quantitative reverse transcription-PCR in the relatives of the index case without WT1 mutation (the index case’s father [Figure 3], the index case’s uncle, and the index case’s maternal grandparents [not shown]) showed a +KTS/−KTS ratio in the normal range between 1.40 and 1.80 (12,14). In contrast, the index case and her mother (Figure 3) had a low ratio (0.40 and 0.34, respectively), within the range reported in Frasier syndrome (12–14) (unpublished observations). These altered ratios were found in renal and ovarian biopsies in the index case’s mother and in a lymphoblastoid cell line in the index case.

Comparison of the +KTS/−KTS ratios of the index case and her mother is, however, considered valid for two reasons. First, we have measured the same +KTS/−KTS ratios (1.5 to 2) in several lymphoblastoid cell lines and kidney samples previously used as controls. Second, the results cannot be explained by WT1 imprinting. This event has not been reported in human kidney (21,22). It can occur in an individual-specific manner in lymphocytes (22), but a WT1 allele imprinting can be excluded in the index case’s lymphocytes since the observed
WT1 are required to achieve normal male sexual characters. A 46, XY individual. This indicates that interactions between abnormal testis determination and pseudohermaphroditism in a 46, XX individual. In contrast, the same mutation leads to not seem to have consequences in the ovarian development in

<table>
<thead>
<tr>
<th>Father (normal)</th>
<th>+KTS/-KTS</th>
<th>1.50</th>
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<table>
<thead>
<tr>
<th>Daughter</th>
<th>+KTS/-KTS</th>
<th>0.40</th>
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<table>
<thead>
<tr>
<th>Mother</th>
<th>+KTS/-KTS</th>
<th>0.34</th>
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104 bp 113 bp

Figure 3. Quantification of WT1 +KTS/-KTS isoforms in the father (no WT1 mutation), the mother (1228+5 G→A heterozygous mutation), and their daughter (index case) (1228+5 G→A heterozygous mutation). See text for details. The isoform ratio was calculated as the ratio of the area under the curve of the two peaks separated by 9 bp. The presented values are means of at least three experiments.

altered +KTS/-KTS ratio is approximately 0.50, in agreement with the theoretical ratio resulting from a WT1 biallelic expression of a heterozygous splice site mutation abolishing the +KTS transcript (13,14).

This case report of familial transmission of a WT1 splice site mutation reveals a high phenotypic polymorphism. In fact, the index case’s mother has typical FSGS in the absence of significant impairment in gonadal functions, while the index case presents with early-onset diffuse mesangial sclerosis and XY pseudohermaphroditism typical of DDS, but without Wilms’ tumor at last follow-up. We could detect neither mutation mosaicism in the index case’s mother nor additional WT1 mutations in the index case, mutations that could explain such phenotypic polymorphism. The lack of abnormality of external and internal genitalia and the conserved reproductive function of the index case’s mother show that although WT1 is expressed in both testis and ovary, WT1 splice site mutation does not seem to have consequences in the ovarian development in a 46, XX individual. In contrast, the same mutation leads to abnormal testis determination and pseudohermaphroditism in a 46, XY individual. This indicates that interactions between WT1 isoforms at a precise ratio and male-determining genes are required to achieve normal male sexual characters.

The distinct pattern of the glomerular disease in the index case and in her mother is striking. It might be argued that FSGS, i.e., one of the most common glomerulopathies, was not caused by the WT1 mutation. The onset of proteinuria during childhood strongly favors a causal link between WT1 mutation and the glomerulopathy in the index case’s mother. The distinct glomerular diseases observed in the index case and in her mother lead to a discussion of two important issues. First, what is the molecular basis of the renal phenotypic polymorphism? Second, could WT1 mutations contribute to break down the heterogeneous entity referred to as “primary” FSGS?

Considering that in dominant genetic diseases normal variants with low expression may modulate the clinical expression of mutant alleles (23), we determined the +KTS/-KTS isoform ratio in several members of the kindred. The two affected patients had a similar low ratio, whereas the nonaffected individuals had a normal ratio. We also measured a comparable low +KTS/-KTS ratio of 0.54 from lymphoblastoid cell line RNA in a 13-yr-old 46, XX female patient harboring the 1228+4 C→T mutation (data not shown). This patient, in whom puberty had developed normally (patient P4 in the study by Jeanpierre et al. [20]), presented with isolated diffuse mesangial sclerosis. Thus, the renal phenotypic polymorphism associated with these mutations does not seem to be linked with the low expression of the controlateral allele. Furthermore, that the same +KTS/-KTS ratio was observed in the index case and her mother argues against mosaicism in the mother. It may be concluded from our data that a low +KTS/-KTS ratio is associated with podocyte abnormalities regardless of the sexual chromosomal background, but that other genetic or nongenetic factors must account for the varying expression of the renal disease ranging from the mild FSGS phenotype to the severe diffuse mesangial sclerosis phenotype. These factors may also explain several unexpected findings, including: (1) the transmission of the WT1 exon 9 R394W mutation from a phenotypically unaffected father to his child presenting with DDS (24); (2) the fact that a few patients with DDS-like disease and exon 9 missense mutation have FSGS (25) instead of diffuse mesangial sclerosis; and (3) the varying renal disease severity in a kindred with FSGS mapping to chromosome 19q13 (8).

WT1 downregulates several genes involved in glomerular development and in fibrogenesis, including those encoding PAX-2, EGR1, transforming growth factor-β, platelet-derived growth factor-A, epidermal growth factor receptor, insulin-like growth factor (IGF)-II, IGF receptor, and novH (an IGFBP-related protein) (26). +KTS and −KTS isoforms have different DNA binding properties. The renal polymorphism that we observed might be related to different activation levels of WT1 target genes by WT1 mutants due to polymorphism in the WT1 target gene or promoter sequences.

The second issue worthy of discussion concerns the prevalence of genetic abnormalities in patients with “primary” FSGS. This report suggests that cases of “primary” FSGS might be caused by WT1 splice site mutation, especially in female patients. The index case’s mother would indeed have been diagnosed as a primary FSGS if she had not become pregnant. There is only one report of a 46 XX patient with possible Frasier syndrome (14). Interestingly, this 37-yr-old woman appeared to have little, if any, impairment of gonadal development and presented with the same 1228+5 G→A
mutation as our patients. The rarity of 46 XX karyotypes in Frasier syndrome is most likely due to underdiagnosis, since these female patients suffer primarily from nephrotic syndrome and later on from renal insufficiency, without significant gonadal impairment. WT1 splice site mutations should be sought in children and adults presenting with apparent primary FSGS. However, it is not possible to apply the expensive, tedious mutation detection methods that are currently available to all patients suffering from primary FSGS. Our case report suggests that the best candidates for such an exacting investigation are females with early-onset proteinuria, regardless of the sexual chromosomal background.

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