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## Frasier Syndrome: A Cause of Focal Segmental Glomerulosclerosis in a 46,XX Female

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**Abstract.** The description of Frasier syndrome until now has been restricted to XY females with gonadal dysgenesis, progressive glomerulopathy, and a significant risk of gonadoblastoma. Mutations in the donor splice site in intron 9 of the Wilms' tumor (WT1) gene have been shown to cause Frasier syndrome and are distinct from WT1 exon mutations associated with Denys-Drash syndrome. The WT1 gene, which is essential for normal kidney and gonadal development, encodes a zinc finger transcription factor. The intron 9 alternative splice donor site mutation seen in Frasier syndrome leads to loss of three amino acids (+KTS isoform), thus disrupting the normal ratio of the +KTS/−KTS isoforms critical for proper gonadal and renal development. This study examines two sisters with identical intron 9 mutations. The proband carries a classic

diagnosis of Frasier syndrome with 46,XY gonadal dysgenesis, whereas her sister has progressive glomerulopathy but a 46,XX karyotype and normal female development. This indicates that the proper WT1 isoform ratio is critical for renal and testicular development, but apparently does not affect either ovarian development or function. It is proposed that the clinical definition of Frasier syndrome should be broadened to include 46,XX females with normal genital development and focal segmental glomerulosclerosis associated with a WT1 intron 9 donor splice site mutation. Nephrologists need to consider the possibility of this heritable syndrome in evaluation of females with focal segmental glomerulosclerosis and to consider their risk for gonadal malignancy, as well as the risk for kidney disease, gonadal dysgenesis, and malignancy in their offspring.

Frasier syndrome is a rare disease in phenotypic females who develop nephrotic-range proteinuria in childhood, have idiopathic focal segmental glomerulosclerosis (FSGS), and progress to end-stage renal disease by the second or third decade of life (1). These patients are often diagnosed when evaluated for primary amenorrhea since, until the current report, the classic description of Frasier syndrome patients has been limited to XY females. They have a normal sex-determining gene on the Y chromosome (SRY) but have gonadal dysgenesis and streak gonads with a significant risk of gonadoblastoma.

Recently, mutations of the Wilms' tumor (WT1) gene have been shown to cause Frasier syndrome (2). The WT1 gene is located on chromosome 11p13 and encodes a zinc finger transcription system essential for normal renal and genital development and for tumor suppression. The mutation of WT1 in Frasier syndrome is distinct from the WT1 exon mutations associated with Denys-Drash and the chromosome 11 deletion

known to cause WAGR syndrome (3). In Frasier syndrome, the mutations reported thus far are within the intron 9 donor splice site of WT1. These are predicted to lead to an imbalance of the ratio of isoforms resulting from an alternative splice site located between the third and fourth zinc fingers of WT1 (2,4).

Here we report two sisters with Frasier syndrome. The proband has classical Frasier syndrome with FSGS and 46,XY gonadal dysgenesis. However, her sister also has FSGS but has a 46,XX karyotype and normal female gonadal development. This is the first fully characterized demonstration of Frasier syndrome in a genetic female. It demonstrates that the FSGS of Frasier syndrome is caused by the WT1 mutation regardless of the patient's genetic sex; however, in contrast to the picture seen in XY individuals, patients with a XX karyotype have apparently normal ovarian development. Also, some cases of FSGS in otherwise normal females may possibly be due to Frasier syndrome, especially when more than one girl in a family is affected.

### Materials and Methods

#### Patients

The proband had asymptomatic 4+ proteinuria noted during a routine physical examination at age 7. She had no edema. Her 24-h urinary protein excretion was 3.5 g and serum albumin was 3.5 g/dl. Serum creatinine was 0.5 mg/dl and cholesterol 301 mg/dl. She failed to respond to prednisone, and a renal biopsy demonstrated FSGS in two of 44 glomeruli (Figure 1A).

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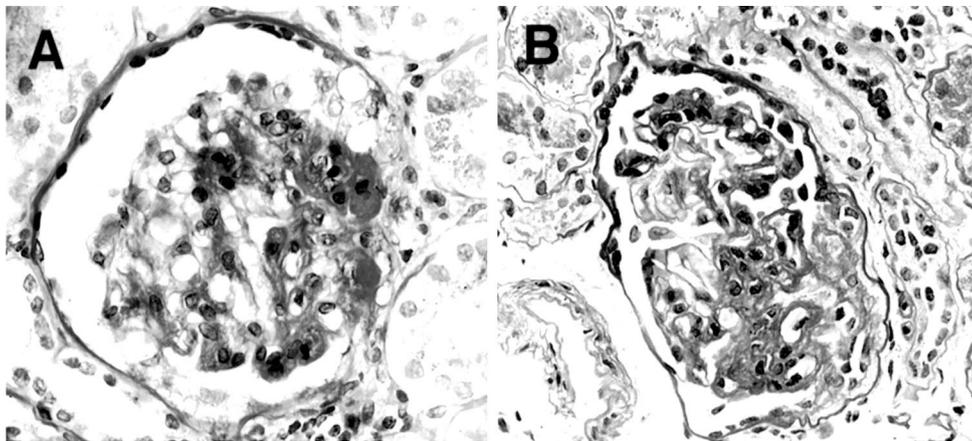


Figure 1. Section from renal biopsy of proband (A) and her sister (B) showing segmental glomerulosclerosis. Magnification,  $\times 400$ .

She had no response to an 8-wk course of oral cyclophosphamide. Proteinuria ranging from 2 to 5 g in 24 h persisted, and angiotensin-converting enzyme inhibitors were prescribed. At age 16, evaluation of primary amenorrhea revealed a 46,XY karyotype (with presence of SRY by fluorescence *in situ* hybridization), Tanner II breast development and unambiguous female genitalia. Magnetic resonance imaging revealed a small uterus with no detectable gonads. Laparoscopy revealed small bilateral testosterone-producing hilar cell adenomas; no gonadal tissue was seen. At age 18, serum creatinine was 1.0 mg/dl and urine protein to creatinine ratio was 6.7.

Her sister had asymptomatic 4+ proteinuria diagnosed at age 5 yr. Renal biopsy also showed FSGS (Figure 1B). No therapy other than angiotensin-converting enzyme inhibition was given. She had menarche at age 11 and has regular menses. Genitalia are unambiguous female with normal uterus and adnexae on ultrasound. Karyotype is 46,XX. Currently at age 16 serum creatinine is 2.4 mg/dl with a urine protein to creatinine ratio of 9.2.

#### Laboratory Studies

DNA samples obtained from the proband, her sister, and her mother were screened for mutations in WT1 by direct sequencing of exons 7 to 10 (zinc finger domains) and exon-intron junctions (2). Genomic DNA was extracted from blood lymphocytes using standard techniques. PCR amplification of exon 9 was performed on genomic DNA using the primers WT9A sense (5'-TAGGGCCGAGGCTAGACCTTCTCTGT-3') and WT9B antisense (5'-ATTCCCTCATCACAAATTCATTC-3'). The PCR conditions were 94°C for 30 s, 47° for 30 s, and 72° for 1 min for 35 cycles. After visualization of the PCR products by gel electrophoresis, column purification was performed with Fisher Clean (Fisher Scientific, Pittsburgh, PA). Samples were sequenced with ABI Prism Dye Sequencing kit (Perkin Elmer, Norwalk, CT) according to the manufacturer's instructions. Sequencing was performed on both sense and antisense strands to confirm the presence of a mutation.

#### Results

Direct sequencing of the exon 9 exon/intron junction revealed a cytosine-thymidine transition in one allele at position +4 of the splice donor site within intron 9 (Figure 2). This

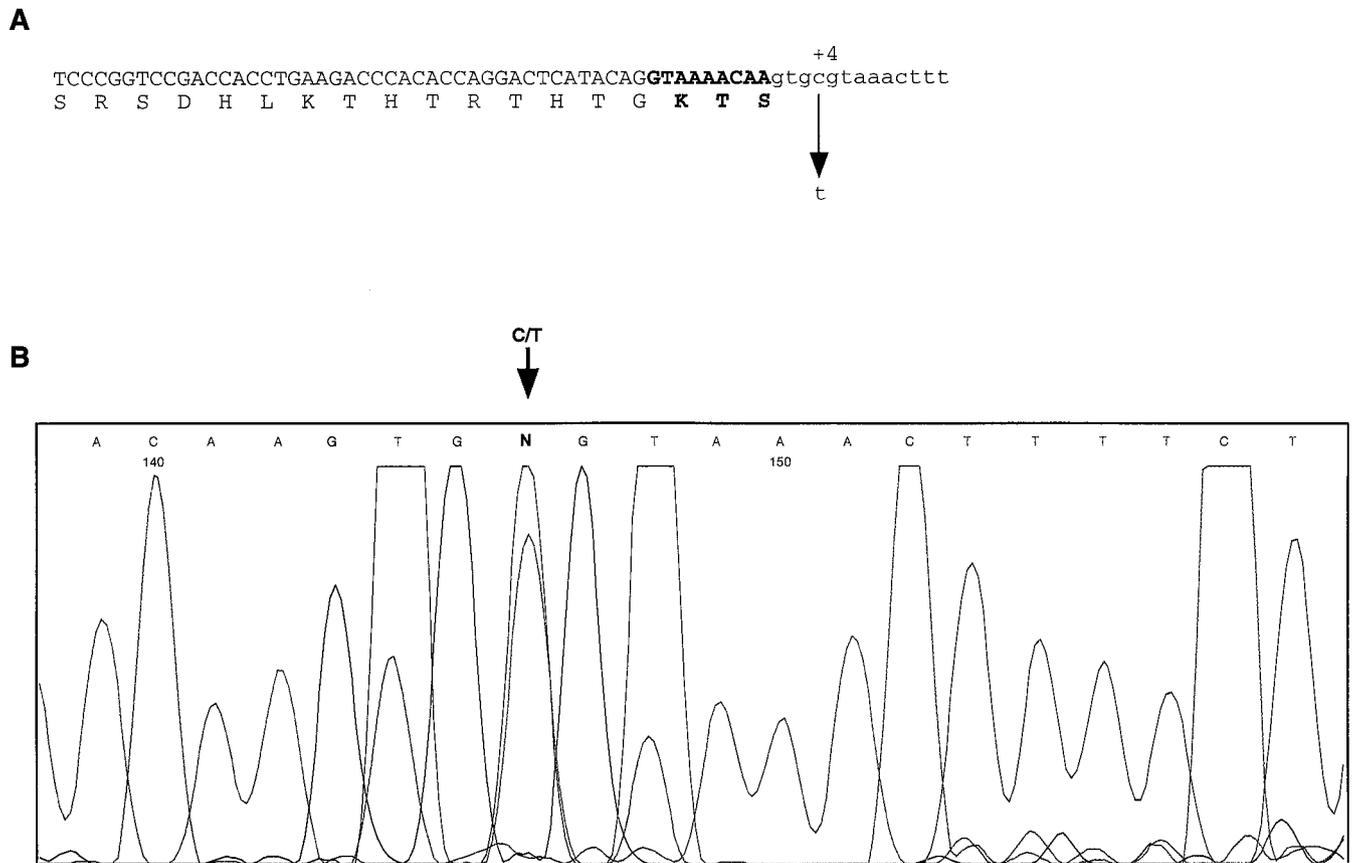
mutation was detected in both sisters but not in their normal mother. This change is predicted to alter the alternative splicing of the KTS (K = lysine, T = threonine, S = serine) motif and in a previous study the same transition was associated with Frasier syndrome and was shown to cause a diminution of the +KTS/–KTS isoform ratio (2,4).

#### Discussion

These two sisters, one 46,XY and the other 46,XX with FSGS have an identical mutation affecting alternative splicing of the WT1 gene. Consequently, the definition of Frasier syndrome should be broadened to include 46,XX females with normal genital development and FSGS associated with a WT1 intron 9 donor splice site mutation.

The origin of the mutation inherited by these two sisters remains speculative. Both parents are reportedly without renal disease, and there is no history of infertility or malignancy. The mother's blood did not show the mutation and, unfortunately, blood from the father was not able to be obtained. Gonadal mosaicism on the part of one of the parents is a well-described genetic phenomenon and is the likely explanation for this mutation appearing in two sisters.

WT1 is highly expressed in the developing kidney in the nephrogenic zone and proximal tubules (3). It is later expressed in the glomerular podocytes, suggesting that it may be essential for normal terminal differentiation of the glomerulus and normal glomerular function (5). Mutations in WT1 cause other renal syndromes. Denys-Drash (male pseudohermaphroditism, early renal failure, and predisposition to Wilms' tumor) (6) is caused by a dominant negative mutation in exon 9 (7,8). Thus, one allele is producing a normal WT1 protein product while the mutated allele is making a defective product responsible for the diffuse mesangial sclerosis, severe proteinuria, and early renal failure seen in Denys-Drash. This abnormal WT1 product hypothetically lacks a renal tumor suppressive effect. This may lead to Wilms' tumor formation in any cell in which the function of the remaining WT1 allele is lost. Contiguous gene deletion of 11p13, which includes the WT1 gene, results in WAGR syndrome (Wilms' tumor, aniridia, genital abnormal-

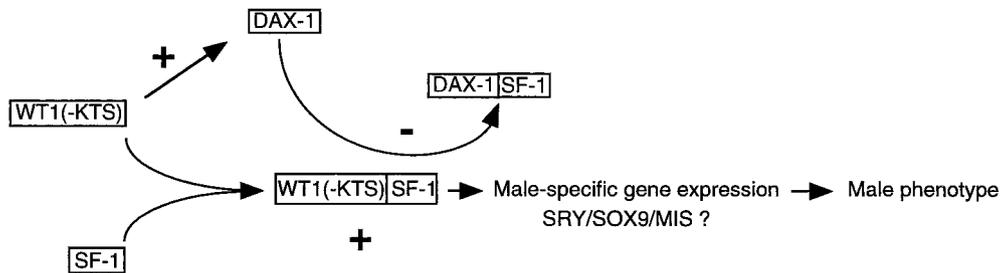


**Figure 2.** (A) Nucleotide and amino acid sequence of WT1 exon 9 and exon/intron junction. Cytosine to thymidine transition at position +4 of the alternative splice donor site reduces formation of the KTS isoform. (B) Direct sequencing of the exon 9 exon/intron junction revealing a cytosine-thymidine transition in one allele at position +4 of the splice donor site in the proband and her sister.

ities, and mental retardation) (9). The absence of the renal tumor suppressive effect of the WT1 protein predisposes to the high frequency of Wilms' tumor seen in WAGR. In contrast, in Frasier syndrome, the mutations cause an imbalance of the KTS isoforms rather than formation of a mutated protein, and the Wilms' tumor suppressive effect is intact (2,4). Although Barbosa *et al.* have described a nephroblastoma in one Frasier syndrome patient, Wilms' tumor is not commonly seen in this syndrome (10). Perhaps the proteinuria that is the first symptom of Frasier syndrome is also caused by an as yet not understood defect in the podocyte resulting from the KTS isoform imbalance.

Analysis of WT1 gene mutations in Frasier patients is helping to elucidate the role of this gene in normal gonadal development. WT1 knockout mice lack gonads in both sexes, demonstrating that the presence of the gene is necessary for the formation of the genital ridge prior to the differentiation of the gonad into ovaries or testes (11). Our patients provide the first genetic evidence that the appropriate ratio of +KTS/−KTS isoforms is critical for testis determination but has no apparent effect on either ovarian development or function. How these data fit into genetic models of sex determination remains speculative (Figure 3). However, recent experiments indicate that the WT1 can form a heterodimer with another protein associated with gonad formation, the orphan nuclear receptor

SF-1 (steroidogenic factor-1) (12). SF-1 is located on human chromosome 9q34. Mutations in SF-1 cause 46,XY gonadal dysgenesis and also adrenal failure, which indicates that SF-1, like WT1, plays a key role in testis formation (13). The −KTS isoform has a greater affinity for the SF-1 protein, and this heterodimer can modulate the expression of mullerian inhibiting substance *in vitro*. Another gene associated with 46,XY gonadal dysgenesis is DAX-1 (for DSS [dosage-sensitive sex reversal]-AHC [adrenal hypoplasia congenita] critical region on the X chromosome gene 1) (14). Like SF-1, DAX-1 encodes an orphan nuclear receptor localized on Xp21.3 that is necessary for the development of the adrenal gland (15). Although loss of function mutations in the gene do not appear to affect either testis or ovarian development, two copies of the gene (*e.g.*, a man with an Xp21 duplication) impair testis determination, resulting in gonadal dysgenesis and a 46,XY female phenotype (15,16). Therefore, although DAX-1 does not appear to be necessary for sex determination, overexpression of the gene product can cause gonadal dysgenesis. WT1 KTS isoforms can modulate the expression of murine DAX-1 expression constructs *in vitro* (17). Taken together, these data suggest a direct, and an essential role for the WT1 KTS isoforms in mammalian testis determination. Changes in the relative amounts of WT1 isoforms in the genital ridge at the moment of sex determination could result in: (1) inappropriate



**Figure 3.** A hypothetical model of mammalian sex determination. The WT1-KTS isoform forms a heterodimer with SF-1 resulting in male-specific gene expression. This may include upregulation of SRY, SOX9, and mullerian-inhibiting substance (MIS). The DAX-1 protein also has the capacity to form a heterodimer with SF-1. Alterations in the ratio of WT1 +KTS and –KTS isoforms, as occurs in Frasier syndrome, could result in the absence or reduced expression of male-specific genes leading to a female phenotype. This may be caused by overexpression of DAX-1, resulting in competition with the WT1 protein for SF-1 binding, a situation analogous to that observed in 46,XY females with two copies of the DAX-1 gene.

interactions with the SF-1 gene product impairing testis determination; and/or (2) an overproduction of the DAX-1 gene product leading to gonadal dysgenesis, a situation analogous to that observed in patients with a duplication of Xp21.3, which contains the DAX-1 gene (reviewed in reference 16).

We propose that the clinical definition of Frasier syndrome should be broadened to include 46,XX females with normal genital development and FSGS associated with a WT1 intron 9 splice site mutation. The XX patient is progressing to end-stage renal disease as is expected in patients with Frasier syndrome. The risk for gonadoblastoma in this young woman is unknown but probably is low because of her clinically normal ovarian function. Rather, it is thought to be the presence of streak gonads and a Y chromosome in classical XY Frasier patients that predisposes to gonadoblastoma. We expect transmission of the mutant WT1 gene to be autosomal dominant. Therefore, the XY offspring of our XX patient will have a 50% likelihood of being phenotypic female with Frasier syndrome. The XX offspring are likely to have a 50% risk of FSGS with normal ovarian development.

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