

Lack of Puberty Despite Elevated Estradiol in a 46,XY Phenotypic Female with Frasier Syndrome

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Abstract. Frasier syndrome is characterized by slowly progressive nephropathy, male pseudohermaphroditism, streak gonad, and high risk of gonadoblastoma development. Here we report a case of a 46,XY phenotypic female with Frasier syndrome, who was under hemodialysis. While her serum estradiol level was gradually increasing annually, gonadotropin level was constantly extremely high, and her appearance was still prepubertal. She was heterozygous for a novel guanine>adenine point mutation at position +1 of the splice donor site within intron 9 (IVS 9 + 1G>A) of the Wilms' tumor 1 gene. The possibility of this disease should be taken into consideration whenever we encounter a patient with steroid-resistant nephrotic syndrome and delayed puberty.

Key words: Frasier syndrome, Wilms' tumor 1 gene, Steroid-resistant nephrotic syndrome, Pseudohermaphroditism, Hemodialysis

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THE Wilms' tumor gene, WT1, encodes a protein with four C-terminal zinc-finger motifs, which is thought to have tumor suppressor activity and to play an important role in the development and subsequent normal function of the urogenital system [1]. Defects in the *WT1* gene account for only a minority of Wilms' tumor (WT) cases, but have been demonstrated to cause the WAGR syndrome (OMIM 194072), Denys-Drash syndrome (DDS, OMIM 194080) and Frasier syndrome (FS, OMIM 136680). The WAGR syndrome, which includes WT, aniridia, genital anomalies and mental retardation, is caused by constitutional deletion of one copy of the *WT1* gene [2]. DDS, which consists of gen-

ital ambiguity of variable degree and early onset renal failure (RF) due to diffuse mesangial sclerosis with high risk of developing WT, is caused by heterozygous mutations of the *WT1* gene [3]. FS, which comprises late onset RF due to nonspecific focal and segmental glomerular sclerosis, and complete gonadal dysgenesis, is caused by heterozygous mutation occurring at an alternative splice site at intron 9 between the region encoding the 3rd and 4th zinc finger motifs [4, 5]. The risk of developing WT is much lower in FS than in DDS, but patients with Frasier syndrome have a higher risk of gonadoblastoma arising from the streak gonads.

Here we report a 46,XY female patient with FS under hemodialysis. Genetic analysis confirmed the diagnosis, showing a mutation at the alternative splice site.

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Case Report

We report the case of a Japanese phenotypic female with male pseudohermaphroditism. She was a ninth child, and had been raised in an orphanage from 9 years old due to family problems. At the age of 2, she was found to have proteinuria at first, and developed nephrotic syndrome that was resistant to corticosteroid therapy. Unfortunately, renal biopsy was not performed. Because of low compliance in hospital visits, her renal function declined rapidly. She manifested generalized seizures twice due to uremia, and emergent hemodialysis was initiated at 6 years and 11 months old. Subsequently, she was started on continuous ambulatory peritoneal dialysis at the age of 7 due to terminal renal insufficiency. Two years later, hemodialysis was initiated because of frequent peritonitis. Since then, she has been anuric and on hemodialysis three times a week using a dialyzer, waiting for cadaver renal transplantation. She was administered with a variety of medications, including antihypertensive medicine, calcitriol and erythropoietin, as well as antiepileptic drugs. As she grew older, short stature was gradually apparent. As her height became below -2.5 SD for her age, growth hormone (GH) treatment was started at the age of 10. Thereafter, GH treatment (0.35 mg/kg/week) had been continued until the age of 16 years, with good response of height gain.

At the age of 15 years and 11 months old, she was first referred to us for consultation concerning delayed puberty. She had no spontaneous secondary sexual development. Blood examination revealed primary ovarian dysfunction with elevated levels of FSH and LH. Chromosome analysis from peripheral lymphocytes demonstrated 46,XY. At the age of 16, further evaluation was carried out.

Her height was 151.4 cm (-1.17 SD for standard Japanese females) and her weight was 38.1 kg (-1.8 SD). Arm span was 151.5 cm, and % body fat was 14.5% . Blood pressure was $143/85$ mmHg. She had complete prepubertal appearance, cubitus valgus and normal female external genitalia, with a vagina that was 6 cm length as determined by pelvic examination.

Laboratory investigations are summarized in Table 1. Elevated urea and creatinine levels and metabolic acidosis were compatible with the end stage renal failure with anuria. Basal serum gonadotropin levels were extremely elevated: FSH 341.0 mIU/ml (normal laboratory range for an adult female: follicular phase

Table 1. Laboratory data at 16 years old

WBC	4880/ μ L
RBC	338×10^4 / μ L
Hb	9.9 g/dL
PLT	23.2×10^4 / μ L
Na	139 meq/L
K	3.9 meq/L
Cl	101 meq/L
BUN	72 mg/dl
Crn	12.5 mg/dl
UA	8.2 mg/dl
Ca	4.8 meq/L
IP	5.2 mg/dL
Mg	2.6 meq/L
AST	10 IU/L
ALT	5 IU/L
ALP	969 IU/L
LDH	178 IU/L
ChE	3130 IU/L
TC	188 mg/dl
TP	7.1 g/dl
Alb	3.9 g/dl
pH	7.261
pCO ₂	40.4 mmHg
HCO ₃ ⁻	17.5 meq/L
BE	-8.5 meq/L
TSH	0.7 μ U/ml
FT4	0.9 ng/dl
FT3	3.2 pg/ml
PRL	10.5 ng/ml
ACTH	39 pg/ml
Cortisol	5.3 μ g/dl
IGF-1	554 ng/ml
IGFBP-3	8.08 μ g/ml
FSH	341.0 mIU/ml
LH	118.4 mIU/ml
E2	49 pg/ml
Prog	0.4 ng/ml
T	0.2 ng/ml
Free T	<2.0 pg/ml
DHEAS	188.7 μ g/dl
CEA	2 ng/ml
AFP	<5 ng/ml
hCG	<3 mIU/ml
CA125	9 U/ml
PTH	353 pg/ml
1,25(OH) ₂ D	23 pg/ml
BAP	396 U/L
NTX	2560 nmolBCE/mmolCr

4.5 – 11.0 ; pre-ovulatory peak 3.6 – 20.6 mIU/ml; luteal phase 1.5 – 10.8 mIU/ml); LH 118.4 mIU/ml (follicular phase 1.7 – 13.3 ; ovulatory phase 4.1 – 68.7 mIU/ml; luteal phase 0.5 – 19.8 mIU/ml). Estradiol (E2) was 49 pg/ml (follicular phase 10 – 200 ; ovulatory phase 103 – 366 ; luteal phase 14 – 251 pg/ml), and progesterone (Prog) was 0.4 ng/ml (follicular phase 0.1 – 1.5 ;

luteal phase 2.5–28 pg/ml). The previous data of the serum sex hormone before our survey were as follows: E2 <10 pg/ml at 13 years and 11 months old; FSH 360.5 mIU/ml, LH 135.7 mIU/ml at 14 years and 4 months old; FSH 457.5 mIU/ml, LH 119.9 mIU/ml, E2 19 pg/ml at 15 years and 4 months old; FSH 322.2 mIU/ml, LH 116.6 mIU/ml, E2 34 pg/ml at 15 years and 10 months old. While her serum estradiol levels were gradually increasing annually, the levels of gonadotropin were constantly extremely high. The level of dehydroepiandrosterone sulfate (DHEAS) was 188.7 µg/dl, suggesting that adrenarche has already started. Serum concentration of total testosterone (T) was 0.2 ng/ml and free testosterone (free T) was under the limit of detection, which was compatible with phenotypic female with no signs of virilization. Serum concentrations of PRL, TSH, free thyroxine (FT4) and free triiodothyronine (FT3) were normal. Serum concentrations of tumor markers including carcinoembryonic antigen (CEA), α -fetoprotein (AFP), human chorionic gonadotropin (HCG), carbohydrate antigen (CA125) were measured, but no significantly increased levels were found. Insulin-like growth factor 1 (IGF-1) (554 ng/ml) was normal for her age (313–759 ng/ml); however, insulin-like growth factor binding protein 3 (IGF-BP3) was elevated to 8.08 µg/ml for her age (2.43–5.70 µg/ml) under GH therapy. Parathyroid hormone (PTH) was elevated to 353 pg/ml, and bone alkaline phosphatase (BAP) and urinary type I collagen cross-linked N-telopeptide (NTX) were also elevated to 396 U/l and 2560 nmolBCE/mmolCr, respectively. As she was completely anuric, we compared the laboratory data before and after hemodialysis at 16 years and 7 months old (Table 2). The endocrinological data were not affected by hemodialysis without the effect of removal of water.

Pelvic ultrasonography showed the presence of a prepubertal uterus and no detectable gonads. Abdominal MRI showed a small uterus, no apparent gonads and atrophic kidneys with multiple cysts. Bone age as estimated by the Japanese TW-2 RUS method was 9 years and 6 months old at the chronological age of 16 years and 1 month. Bone mineral density (BMD) was evaluated by dual-energy X-ray absorptiometry (LUNAR, DPX-L). BMD of the lumbar spine (L2–4) was 0.915 g/cm², which was comparable to that of other girls aged 11 years old. The association of male to female sex reversal with early onset RF prompted us to perform a molecular analysis of the *WT-1* gene.

Table 2. Laboratory data before and after hemodialysis at 16 years and 7 months old

		before dialysis	after dialysis
FSH	mIU/mL	382.8	404.8
LH	mIU/ml	149.4	174.7
E2	pg/ml	38	37
Prog	ng/ml	0.4	1.1
T	ng/ml	0.35	0.34
IGF-1	ng/ml	405	475
DHEAS	mg/dl	167.2	173.6
Na	meq/L	136	141
K	meq/L	5.3	4.9
BUN	mg/dl	83	38
UA	mg/dl	7.8	3.7
Cre	mg/dl	12.7	6.9
Ca	meq/l	4.5	4.8
P	mg/dl	5.3	4.5
ALP	IU/l	362	424
Alb	g/dl	4.0	4.8

Genetic Analysis

We sequenced the genomic DNA in the region of the *WT1* gene where mutations have been identified in other patients with FS. The Institutional Review Board approved this study protocol, and written informed consent for genomic analysis was obtained from the father and the patient. Genomic DNA was extracted from white blood cells using standard techniques. PCR amplification of exons 8 and 9 and the exon/intron boundaries was accomplished using the following sets of specific primers: exon 8, sense, 5'-TTAATGAGA TCCCCTTTTCC-3' and antisense, 5'-TGTTCTGTT GGTTCGCCAGGG-3'; exon 9, sense, 5'-TGGCAGG AAATGCTGGGCTCC-3' and antisense, 5'-AAGAT AGCCACGCACTATTCC-3' [6]. The PCR conditions used were: 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, annealing at 55°C for 30 sec and 72°C for 30 sec; final extension at 72°C for 5 min. Following purification of the PCR products from low-melting agarose gel, direct sequencing was carried out using an automatic sequencer (Model 377, Applied Biosystems, Foster City, CA).

We found that the patient was heterozygous for a guanine>adenine point mutation at position +1 of the splice donor site within intron 9 (IVS9 + 1G>A) (Fig. 1). The correlation of male karyotype and female phenotype (complete sex reversal), severe nephropathy, streak gonads and genetic analysis confirmed the diagnosis of FS.

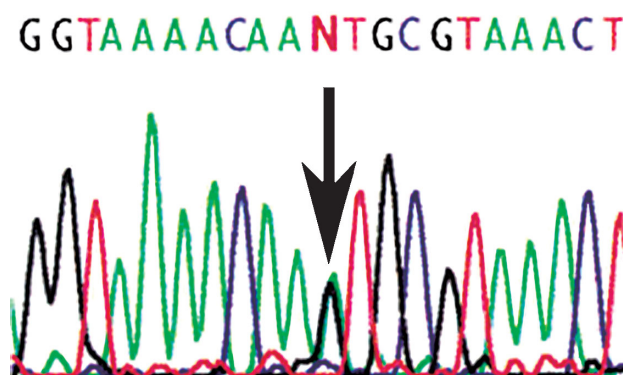


Fig. 1. Chromatogram shows heterozygosity for a guanine>adenine point mutation at position +1 of the splice donor site within intron 9 of the *WT1* gene.

The patient was scheduled for surgical removal of the streak gonads, given the risk of developing gonadoblastoma. However, she suddenly died while bathing at 16 years and 8 months old, and histological analysis of gonads could not be performed.

Discussion

Frasier syndrome is a rare disorder associated with late onset RF due to nonspecific focal and segmental glomerular sclerosis and complete gonadal dysgenesis. In 1964, Frasier *et al.* reported phenotypic female identical twins with XY gonadal dysgenesis with streak gonads, gonadoblastoma, and chronic kidney failure [7]. Similar patients were subsequently described, and in 1987 Moorthy *et al.* collected 6 patients and proposed that these symptoms should be called Frasier syndrome (FS) [8]. FS is caused by mutation in the *WT1* gene, which is located on chromosome 11p13. *WT1* gene encodes a zinc-finger protein that has been implicated as causing a number of disorders in urogenital development [4]. Recently, Wang *et al.* identified a classic mutation in the *WT1* gene in one of the original cases of FS reported in 1964 [9]. The mutations occur within intron 9, affecting a donor splice site [5] that is critical for the expression of an alternatively spliced isoform of the *WT1* protein. These isoforms differ in the presence or absence of a 3-amino acid sequence of lysine, threonine, and serine (KTS). It is believed that imbalance in the expression of these two isoforms underlies the development of FS.

A related disorder, Denys-Drash syndrome (DDS),

which is characterized by Wilms' tumor in association with pseudohermaphroditism and diffuse mesangial sclerosis, is also caused by mutations in *WT1*, typically involving the coding sequence [3]. However, genotype-phenotype correlations are not completely distinct. One family was reported that carried an intron 9 splice site mutation and presented with the DDS phenotype in the child and isolated kidney disease in the mother [10]. Another report demonstrated exon 9 mutations in two patients with Frasier syndrome [11]. These data confirm the variable expressivity of the mutation and the phenotypic overlap between these two disorders. In the case of our patient, a novel mutation, +1 G to A in intron 9 of *WT1*, was detected. The position of this mutation was very close to but different from previously reported mutations in FS [12]. As far as we know, five different mutations have been reported in intron 9 of the *WT1* gene: +2T>C, +4C>T, +5G>A, +5G>T, and +6T>A.

Individuals with FS who have a 46,XY karyotype present with pure intersex state, whereas those with a 46,XX karyotype have normal development of ovary, affirming the less crucial role of *WT1* in normal gonadal development [13]. Since the development of gonadoblastoma is a typical complication of gonadal dysgenesis in patients with male pseudohermaphroditism in the presence of Y chromosome, and more malignant germ cell tumors, such as dysgerminoma, often occur in dysgenetic gonads with gonadoblastoma [14], removal of nonfunctioning streak gonads has been recommended, preferably before a renal transplantation is performed. We could not find previous reports of Frasier syndrome under GH therapy. Since the risk of gonadoblastoma seems to increase in adolescence, we stopped GH therapy and scheduled surgical removal of the streak gonads. We could not evaluate the influence of GH therapy on streak gonads, because our patient died suddenly before the histological examination of gonads could be performed. For example, the risk of occurrence of leukemia in growth hormone-treated patients has been denied so far [15]; however, we cannot completely ignore the possibility of increased malignancy in the high-risk group. Treatment with GH for short stature may not be recommended for patients with Frasier syndrome due to the associated risk of tumor formation.

There are few hormonal studies of gonadotropin levels in FS in the literature. According to the review by Melo *et al.* [16], pubertal and adult patients with

FS had extremely high gonadotropin levels ranging from 53–117 IU/L for LH and from 66–254 IU/L for FSH. All of those patients had dysgenetic gonads. The patient described here also had extremely elevated LH and FSH levels, with a normal adult estradiol level and no pubertal development. This suggests certain possible mechanisms: altered hypothalamic-pituitary-gonadal feedback regulation with resistance to gonadotropin and/or sex hormone, discrepancy of immunoreactive and bioactive hormone, and decreased metabolic clearance rate due to anuria. In our patient the origin of the estrogen could not be clarified, but it may have been from an ovarian element of the dysgenetic gonad, or gonadal tumors including gonadoblastoma. The elevated serum estradiol level was not compatible with her prepubertal appearance. This elevated estradiol may have been due to increased levels

of sex hormone binding protein and low free hormone. Likewise, when IGF-BP3 level is elevated, it decreases the free IGF-1 level and diminishes the growth rate in patients with chronic renal failure. In our case, the growth rate was well maintained under GH therapy but declined abruptly after GH withdrawal, which suggests that the supplemental free IGF-1 was effective for increasing her growth. Another possible explanation was insensitivity of the target organs to estradiol. Due to her unexpected death, further investigations, including measurement of free hormone, histological analysis and estradiol receptor gene analysis, could not be performed.

In conclusion, we should take into consideration the possibility of this disease whenever we encounter a patient with steroid-resistant nephrotic syndrome accompanied by delayed puberty.

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