Case report

Sertoli cell tumor and gonadoblastoma in an untreated 29-year-old 46,XY phenotypic male with Frasier syndrome carrying a WT1 IVS9+4C>T mutation

Sophia Kitsiou-Tzeli*,¹ Maria Deligiorgi*,² Sophia Malaktari-Skarantavou,²
Charalampos Vlachopoulos,³ Spyridon Megremis,¹ Irene Fylaktou,¹
Joanne Traeger-Synodinos,¹ Christina Kanaka-Gantenbein,⁴
Christodoulos Stefanadis,³ Emmanuel Kanavakis¹

¹Department of Medical Genetics, Medical School, University of Athens, Aghia Sophia Children's Hospital, ²Department of Endocrinology and Metabolism, Hippokration General Hospital of Athens, ³1st Department of Cardiology, Medical School, University of Athens, Hippokration Hospital, ⁴Division of Endocrinology, Diabetes and Metabolism, First Department of Pediatrics, Medical School, University of Athens, Aghia Sophia Children's Hospital, Athens, Greece

*These authors contributed equally

ABSTRACT

OBJECTIVE: Frasier syndrome (FS) phenotype in 46,XY patients usually consists of female external genitalia, gonadal dysgenesis, high risk of gonadoblastoma and the development of end stage renal failure usually in the second decade of life. FS is caused by heterozygous de novo intronic splice site mutations of the Wilms' tumor suppressor gene 1 (*WT1*), although a few cases with typical exonic *WT1* Denys-Drash mutations that resemble an FS phenotype have been described. The aim of this study was to present further data on the spectrum of FS phenotypes through the evaluation of a 29-year-old patient with a predominantly male phenotype and coexistence of Sertoli cell tumor and gonadoblastoma. **RESULTS:** Genetic analysis using standard methods for DNA sequencing confirmed FS due to a *WT1* gene mutation, IVS9+4C>T. **CONCLUSIONS:** This very rare case illustrates the natural course of FS over many years due to the neglect by the patient to address his need for follow-up, while adding further data on the spectrum of FS phenotypes associated with IVS9+4 C>T mutations. The coexistence of the rare Sertoli cell tumor and gonadoblastoma emphasizes that early clinical recognition and molecular identification facilitates appropriate patient management, especially with respect to the high risk of gonadal malignancy.

Key words: Frasier syndrome, Sertoli cell tumor, Gonadoblastoma, WT1 gene

Address for correspondence:

Christina Kanaka-Gantenbein, MD, PhD, Division of Endocrinology, Diabetes and Metabolism, First Department of Paediatrics, University of Athens, Aghia Sophia Children's Hospital, Athens 11527, Greece, Fax: +30 210 7779919; email: ganten@hol.gr or chkanaka@med.uoa.gr

Received 18-02-12, Revised 11-04-12, Accepted 19-05-12

INTRODUCTION

Frasier syndrome (FS) was first described in 46,XY monozygotic twins in 1964.¹ The FS phenotype in 46,XY patients usually consists of female external genitalia, gonadal dysgenesis, high risk of gonadoblastoma and development of end stage renal failure usually in the second decade of life. FS is caused by heterozygous de novo intronic splice site mutations in the Wilms' tumor suppressor gene 1 (*WT1*), although a few cases with typical exonic *WT1* Denys-Drash mutations that resemble an FS phenotype have been described.²⁻⁴ Denys-Drash syndrome (DDS) is a rare disorder affecting urogenital development and characterized by the triad of early onset nephrotic syndrome, disorders of sex differentiation and predisposition to Wilms' tumor. FS and DDS have similarities as well as clinical and genetic differences. In DDS, the onset of nephropathy due to diffuse mesangial sclerosis presents in infancy and progresses to end stage renal failure usually before the age of 5 years. In FS, nephropathy due to focal segmental glomerulosclerosis occurs in childhood and progresses to end stage renal failure during the second or third decade of life. In DDS, there is a high risk of Wilms' tumor, whereas in FS the major risk of malignancy is the development of gonadoblastoma. The most frequent *WT1* defects in DDS are missence mutations located in exons 8-9, while FS is predominantly caused by mutations in the donor splice site in intron 9 of the *WT1* gene.^{3,5}

The *WT1* gene is located on chromosome 11p13 and consists of 10 exons that include a zinc finger DNA binding domain. The gene is expressed in a wide variety of embryonic tissues including the mesenchymal cells of the foetal kidney and the stromal cells of the gonads and the spleen.⁶ *WT1* acts as a transcriptional activator or repressor depending on the cellular or chromosomal context.^{7,8} It has four major isoforms, due to the insertion of three amino acids (lysine/threonine/serine, KTS) between zinc fingers 3 and 4 and the insertion of an alternatively spliced 17 amino acid segment encoded by exon 5, resulting from alternative splicing of the pre-mRNA.⁹ FS mutations in intron 9 of the *WT1* gene lead to a change in splicing that results in deficiency of the usually more abundant KTS-positive isoforms and reversal of the normal KTS-positive to negative ratio from 2:1 to 1:2, a balance that is necessary for *WT1* normal function.^{10,11}

We report here a 29-year-old patient with a predominantly male phenotype and coexistence of Sertoli cell tumor and gonadoblastoma. Genetic analysis confirmed Frasier syndrome due to a *WT1* gene mutation, IVS9+4C>T.

MATERIAL, METHODS AND RESULTS

Case Report

The patient, a 29-year-old male, is the only child of non-consanguineous Greek parents. His father was deaf due to a childhood infection and his mother has congenital deafness of unknown origin but is otherwise healthy. At birth, the patient presented with bilateral cryptorchidism, scrotal hypospadias and persistent Mullerian ducts. His karyotype was 46,XY. At the age of 2 years, he underwent orchidopexy on the left side, whereas on the right side the surgeon reported that no testis was found. Later, a surgical repair of hypospadias was undertaken. At the age of 8 years, he underwent removal of the Mullerian structures. During childhood, he experienced several episodes of urinary tract infections due to a diverticulum of the urethra. There was no medical follow-up from age 11 until 29 years, probably because the family wanted to forget the stress provoked by the multiple surgical procedures during early childhood. The patient reported being asymptomatic during this period.

At the age of 29 years he was admitted to the hospital because of severe hypertension (220/100 mmHg) and blurred vision. A complete ophthalmological evaluation demonstrated normal optical acuity and stage 3 hypertensive retinopathy. On physical examination, an extremely low body weight in relation to his height was noted (49 kg, 180cm, respectively, BMI=15.1 kg/m²). The examination of external genitalia revealed poor pubic hair development (Tanner stage 4) and a small penile size with an orthotopic urethral opening. A left testis of approximately 15 ml volume was palpable in the scrotum. There was no right testis palpable in the scrotum, whereas in the right external inguinal ring an ovular lesion of a diameter of less than 1 cm of hard consistency was found.

Laboratory findings on admission

Blood tests revealed end stage renal failure: creati-

nine 9.7 mg/dl, urea 240 mg/dl, serum albumin concentration 2.8 g/dl with total proteins 6.1 g/dl, potassium 4.7 meq/l, sodium 139 meq/l, calcium 8.2 mg/dl and phosphorus 8.9 mg/dl. Protein excretion in 24-hours urine was 350 mg/dl. Anaemia was revealed with anisocytosis, (Hb=9.4 g/dl, Hct=28.9%, MCH=28.8 pg, MCV=88.7), ESR=84 mm at 1hr, TIBC=211 µgr% (lower normal=225 µgr%) and ferritine=417.30 µg/l (upper normal reference value=323 µg/l).

Plasma sulfate dehydroepiandrosterone (S-DHEA) and 17-hydroxy-progesterone (17-OH-PRG) were normal (S-DHEA=1719.5 ng/ml, 17-OH-PRG=1.9 ng/ml). Plasma aldosterone and plasma renin activity in the upright posture were also normal (192.7pg/ml, 8.4µU/ml, respectively). Urine metanephrines, normetanephrines and VMA excretion over a 24-hour period were also normal (84 µg/dl, 128 µg/dl, 1.6 µg/dl, respectively).

The investigation of the hypothalamic-pituitary-testicular axis demonstrated the presence of hypergonadotropic hypogonadism with low normal testosterone (1.82 ng/ml, reference values: 1.66-8.11 ng/ml) and extremely elevated gonadotropins (FSH=148.7 mIU/ml, LH>250 mIU/ml). Thyroid function was normal (TSH=1.15 mIU/ml, FT₃=2.35 pg/ml, FT₄=0.8 mg/dl, Anti-TG Abs: negative, Anti-TPO Abs: negative). Haematological, biochemical and endocrinological findings of the patient at hospital admission are shown in Table 1. Immunological tests (including ANA, c-ANCA, p-ANCA, ENA, Anti-ds-DNA), as well as auditive exams, were normal.

DNA analysis

Due to the concomitant occurrence of genitalia abnormalities and end stage renal failure, Frasier syndrome was suspected and a patient sample (peripheral blood) was referred to the Department of Medical Genetics, Athens University, for DNA analysis to investigate whether the patient had a mutation in the *WT1* gene.¹²

Genomic DNA was extracted from peripheral blood lymphocytes using the commercially available QIAmp DNA Blood Mini kit (Qiagen, Gmbh, Hilden, Germany). Exons 8 and 9 of the *WT1* gene, including 100 bp of the flanking intronic sequences, were amplified by PCR. The primers employed were

Table 1. Haematological, biochemical and endocrinological findings of patient at admission

	Patient	Normal values
Haematology count		
Haemoglobin (g/dl)	9.4	12-18
Haematocrit (%)	28.9	37-52
Mean cell volume (fl)	88.7	80-99
Mean cell haemoglobin (pg)	28.8	27-31
TIBC (µg%)	211	225-480
Ferritin (µg/l)	417.3	18.7-323
Renal function		
Creatinine (mg/dl)	9.7	0.70-1.50
Urea (mg/dl)	240	10-55
Serum albumin (g/dl)	2.8	3.50-5.00
Total protein (g/dl)	6.11	6.00-8.40
Potassium (meq/l)	4.7	3.20-5.20
Sodium (meq/l)	139.1	130-150
Calcium (mg/dl)	8.2	8.50-10.50
Phosphorous (mg/dl)	8.91	2.50-5.00
24hour urine protein mg/dl)	350	<100
Adrenal function		
Plasma sulphate dehydroepiandrosterone (S-DHEA) (ng/ml)	1719.5	800-5600
17-hydroxy-progesterone (17-OH-PRG) (ng/ml)	1.9	0.5-2.9
Metanephrines, 24-hour Urine (µg/dl)	84	52-341
Normometanephrines, 24-hour Urine (µg/dl)	128	88-444
VMA, 24-hour Urine (µg/dl)	1.6	1.8-6.7
Plasma renin activity, upright posture (µU/ml)	8.4	5.0-50.0
Plasma aldosterone activity, upright posture (pg/ml)	192.7	35.0-300.0
Hypothalamic-pituitary-testicular axis		
Testosterone (ng/ml)	1.82	1.66-8.11
FSH (mIU/ml)	148.7	1.37-13.58
LH (mIU/ml)	>250	1.14-8.75
TSH (mIU/ml)	1.15	0.35-4.95
FT3 (pg/ml)	2.35	1.71-3.70
FT4 (mg/dl)	0.8	0.70-1.48

designed using software available in the public domain (Primer3 <http://primer3.sourceforge.net>) based on published information regarding the *WT1* gene

sequence (NCBI Reference Sequence: NG_009272.1). The oligonucleotide sequences of the forward and reverse primers are: exon 8 forward, 5'cctttaatgagatccccttttc3', exon 8 reverse, 5'ggggaaatgtgggtgtttcc3', exon 9 forward, 5'cctcactgtgccacattgt3' and exon 9 reverse, 5'gcactattcctctctcaactgag3'. The PCR programme consisted of an initial denaturation step at 95°C for 15 min followed by 40 cycles, for exon 8 and 35 cycles, for exon 9, of the following steps: 95°C-1 min, 59°C-1 min and 60°C-1 min for exons 8 and 9, respectively, 72°C-1 min and a final elongation step 72°C-8 min. The amplicons were sequenced directly using the 7-Deaza-dGTP Cyt5/Cy5.5 Dye Primer Cycle Sequencing Kit (Bayer HealthCare, Leverkusen, Germany) sequencing kit in an OpenGene Visgen automated sequencer (Visible genetics, Ontario, Canada) and analyzed using the Unix Based Gene Objects 3.1 software. Following data analysis, a point mutation in intron 9 was identified (IVS9+4C>T).

Clinical Progression

Antihypertensive therapy was initiated and haemodialysis was started immediately via an arteriovenous fistula. Remarkably, concomitant with the completion of laboratory and genetic investigations the patient experienced a painful swelling of the left testis. At that time, a noticeable enlargement of the left testis, leading to a palpable mass of approximately 25 ml volume with hard consistency, was observed. Ultrasound scan showed an enlargement of the left testis with diffuse echogenicity and microcalcifications as well as three hypoechogenic lesions of 21mm, 10mm and 14mm diameter, respectively. Additionally, hydrocele was detected, whereas no ectopic right testis was found. Magnetic resonance imaging further confirmed the enlargement of the left testis with heterogeneity of the signal and disordered architecture, features implying a neoplasm. Additionally, in the right groin an oval shaped lesion measuring 17mm in diameter with hypointensity in T1 sequence and intermediate intensity in T2 sequence was revealed. The presence of a right hypoplastic testis could not be excluded. Computed tomography confirmed that there were no pathological lymph nodes. The right kidney had a maximum diameter of 8 cm and the left kidney a maximum diameter of 7.1 cm. Serum tumor markers are summarized in Table 2. The patient was submitted to bilateral testicular resection. Prosthetic testes

Table 2. Serum Tumor markers

	Patient	Normal values
β-HCG (mIU/L)	23.39	<5
LDH (U/L)	409	120-230
Alpha fetoprotein (ng/ml)	0.85	<10

were implanted at the time of gonadectomy. Sperm preservation could not even be considered in the constellation of highly elevated FSH levels.

Histological analysis performed in the left testis revealed a germ cell tumor with morphological and immunohistochemical features consistent with a Sertoli cell tumor. The cells were arranged in tubules with solid areas, a characteristic that made the differentiation from seminoma challenging. Furthermore, the cells demonstrated clear or slightly eosinophilic cytoplasm, nuclei with atypia, mitosis and a great range of appearance as well as areas of necrosis, calcifications and elements of inflammation. Immunohistochemistry supported the discrimination between Sertoli cell tumor and seminoma. Indeed, the staining disclosed a strong positivity for vimentin in the cytoplasm as well as around the nuclei, positivity for cytokeratin and negativity for placental alkaline phosphatase and c-kit-1, which are all features characteristic of Sertoli cell tumor. Markers for neuroendocrine tumors were negative. Nuclei were positive for Ki 67 at a percentage higher than 40%. The size of the neoplasm (6.5 cm), the increased number of mitotic figures as well as the atypia were all indicative of a malignant progress. The tumor was surrounded by an atrophic testis with hyalinized structures and hyperplastic Leydig cells. In the hypoplastic right testis, a gonadoblastoma measuring 0.5 cm in diameter was identified. Subsequently, the patient received adjuvant chemotherapy with Bleomycin, Etoposide and Cisplatin (Platinol) (BEP), with the prospect of continuing with androgen replacement therapy after the completion of chemotherapy.

DISCUSSION

Gonad and kidney development depends on the appropriate expression of the *WT1* gene, located on chromosome 11p13. Mutations at the intron 9 donor splice site of *WT1* lead to a modification of the relative

amounts of the +KTS/-KTS WT1 protein isoforms and lead to the expression of Frasier syndrome.¹³

In FS, five different mutations have been reported to date at the intron 9 donor splice site: c.1228+2 T>C, c.1228+4 C>T, c.1228+5 G>A, c.1228+5 G>T and c.1228+6 T>A. The mutation detected in our patient (c.1228+4 C>T) is the most frequent mutation in FS, accounting for around 52% of reported cases.^{13,14} It is a hotspot mutation which arises from the potential to deaminate 5-methylcytosine at +4 and +5 CpG dinucleotide positions in intron 9.^{13,15} CpG dinucleotides are reported to have the highest rates of mutability for single-base-pair substitutions.¹⁶

In 46,XY patients, FS usually presents with complete gonadal dysgenesis, streak gonads and male to female sex reversal.¹⁷ The external female phenotype leads to female sex of rearing. To our knowledge, 46,XY phenotypically male FS patients are very rare,^{15,18,19} and amongst those genetically characterized only two have the same mutation as the patient in our report. One patient, an 8-year-old boy with IVS9+4C>T mutation in the *WT1* gene and male sex of rearing, reported by Denamur et al,¹⁸ presented proximal hypospadias, cryptorchidism, renal failure, Wilms' tumor and diaphragmatic hernia. Another study reported a 19-year-old male with IVS9+4C>T mutation in the *WT1* gene, who had an unusual phenotype characterized by normal penile size, perineal hypospadias and end stage renal failure at the age of 19 years, extremely elevated gonadotropin levels, para-testicular leiomyoma, unilateral testicular germ cell tumor, bilateral gonadoblastoma and absence of gonadal dysgenesis.¹⁵ Other *WT1* mutations in three other FS patients with predominantly male genitalia (R390X, F392L and IVS9+5G>A) were reported from Japan.^{20,21} The phenotypic variability among different patients with the same mutation suggests that modifier genes and/or environmental factors may play a role in this variation.¹⁷

In our patient, the constellation of the IVS9 + 4C>T mutation in the *WT1* gene, the XY disorders of sexual differentiation, the onset of nephropathy in the second decade of life and the development of gonadoblastoma as well as Sertoli cell tumor support the diagnosis of Frasier syndrome. Our patient had predominantly undervirilized male external genitalia

that led to a male sex of rearing. He was born with bilateral cryptorchidism and scrotal hypospadias, but after surgical interventions at the age of 2 years he achieved male external genitalia and reportedly experienced normal sexual activity. At the age of 29 years he maintained low normal testosterone secretion with hypogonadism. This observation is in agreement with the patient described by Melo et al, who eventually developed bilateral gonadoblastoma and unilateral germ cell neoplasia.¹⁵ Our patient also developed gonadoblastoma in his cryptorchidic right testis and Sertoli cell tumor in his left testis, suggesting that the latter was preceded by gonadoblastoma, as the presence of gonadoblastoma in the right hypoplastic testis increases the risk for a new primary gonadoblastoma in the left testis, which could be a precursor lesion for the Sertoli cell tumor.^{22,23} However, this hypothesis cannot be confirmed. It is possible that gonadoblastoma is a result of the presence of dysgenic gonads or of differences in the stage at which gonadal development is arrested in Frasier syndrome and Danys-Drash syndrome.²⁴

Sertoli cell tumors have a prevalence ranging from 0.4 to 1.5% of testicular malignancies in adults and up to 4% in children.^{17,25} Gomez-García et al reported a phenotypic male with FS and heterozygous *WT1* mutation IVS9+5 G>A who, after prophylactic orchidectomy at the age of 19 years, showed arrest in spermatogenesis, intratubular germ cell neoplasia of unclassified type (ITGCN) and Leydig cell dysfunction.²⁵ These data support a role of *WT1*+*KTS* in terminal Sertoli cell differentiation and maintenance and also confirmed for the first time in humans that critical levels of *SRY* and *SOX9* are also required for normal Sertoli cell differentiation and subsequent normal spermatogenesis. Ultimately, decreased expression of *WT1* + *KTS*, *SRY* and *SOX9* may result in malignant transformation of germ cells.

Frasier syndrome and Denys-Drash syndrome are considered to be a continuum, since the two diseases are not always distinct, representing two ends of a spectrum of disorders caused by alteration in the *WT1* gene.^{15,26} In support of the continuum hypothesis are other reports of FS in a few patients with male ambiguous external genitalia,^{15,18,20,21} and also the development of Wilms' tumor in one case.²⁷ Within the clinical follow-up, the risk of Wilms' tumor

in such patients should also be evaluated, although a recent article provided substantial clarification as to the risk of developing Wilms' tumor in *WT1* positive patients.²⁸ According to Chernin et al, patients with *WT1* missense or nonsense mutations have a high risk of developing Wilms' tumor, whereas patients with splice site mutations have a very low risk.²⁸ Despite these findings, bilateral nephrectomy and kidney transplantation is under consideration in our patient. However, the progression of his gonadal neoplasms will play a determinant role in any treatment decision.

Our report presents a very rare case which illustrates the natural course of FS due to follow-up failure over many years. Furthermore, the present case adds new data to the spectrum of Frasier syndrome phenotypes associated with IVS9+4 C>T point mutations by including one more case of male ambiguous genitalia and also the co-existence of the rare Sertoli cell tumor with gonadoblastoma, a feature that further expands the phenotype variation of *WT* mutations. It is emphasized that the early clinical recognition and molecular identification of the syndrome and removal of the dysgenetic gonads is of utmost importance since the syndrome is associated with a high risk of gonadal malignancy.

DECLARATION OF INTEREST AND FUNDING

Declaration of interest: All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research supported.

Funding: This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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