Clinical and Genetic Findings of Five Patients with *WT1*-Related Disorders

clinical case report

Juliana Gabriel R. de Andrade
Mara Sanches Guaragna
Fernanda Caroline Soardi
Gil Guerra-Júnior
Maricilda Palandi de Mello
Andréa Trevas Maciel-Guerra

Grupo Interdisciplinar de Estudos da Determinação e Diferenciação do Sexo (GIEDDS), Faculdade de Ciências Médicas, Universidade de Campinas (Unicamp) (JGRA, GGJ, ATMG); Centro de Biologia Molecular e Engenharia Genética (CBMEG), Unicamp (MSG, FCS, MPM); Campinas, SP, Brasil.

Received in 25/8/2008
Accepted in 14/10/2008

ABSTRACT

Aim: To present phenotypic variability of WT1-related disorders. **Methods:** Description of clinical and genetic features of five 46,XY patients with *WT1* anomalies. **Results:** Patient 1: newborn with genital ambiguity; he developed Wilms tumor (WT) and chronic renal disease and died at the age of 10 months; the heterozygous 1186G>A mutation compatible with Denys-Drash syndrome was detected in this child. Patients 2 and 3: adolescents with chronic renal disease, primary amenorrhea and hypergonadotrophic hypogonadism; patient 2 had a gonadoblastoma. The heterozygous IVS9+4, C>T mutation, compatible with Frasier syndrome was detected. Patient 4: 9-year-old boy with aniridia, genital ambiguity, dysmorphisms and mental deficiency; a heterozygous 11p deletion, compatible with WAGR syndrome was detected. Patient 5: 2 months old, same diagnosis of patient 4; he developed WT at the age of 8 months. **Conclusions**: Constitutional abnormalities of *WT1* cause gonadal and renal anomalies and predisposition to neoplasia and must be investigated in patients with ambiguous genitalia, chronic renal disease and(or) Wilms tumors; primary amenorrhea with chronic renal disease; and aniridia, genital ambiguity and dysmorphisms. **(Arq Bras Endocrinol Metab 2008; 52/8:1236-1243)**

Keywords: Sex differentiation; *WT1* gene; Denys-Drash syndrome; Frasier syndrome; WAGR syndrome

RESUMO

Achados Clínicos e Genéticos de Cinco Pacientes com Anomalias Relacionadas ao Gene *WT1*.

Objetivo: Descrever a variabilidade fenotípica das anomalias relacionadas ao WT1. **Métodos:** Descrição das características clínicas e genéticas de cinco pacientes 46,XY com anomalias no *WT1*. **Resultados:** Paciente 1: Recém-nascido com ambigüidade genital desenvolveu tumor de Wilms (TW) e insuficiência renal crônica (IRC), com óbito aos 10 meses. Detectada a mutação 1186G>A em heterozigose, compatível com síndrome de Denys-Drash. Pacientes 2 e 3: Adolescentes com IRC, amenorréia primária e hipogonadismo hipergonadotrófico; a paciente 2 apresentava gonadoblastoma. Ambas apresentavam mutação IVS9+4, C>T em heterozigose, característica da síndrome de Frasier. Paciente 4: Idade 9 anos, aniridia, ambigüidade genital, dismorfismos e deficiência mental; deleção 11p, compatível com síndrome WAGR foi encontrada em heterozigose. Paciente 5: Dois meses, mesmo diagnóstico do paciente 4, desenvolveu TW aos 8 meses. **Conclusões:** Alterações constitucionais do *WT1* determinam anomalias gonadais, renais e predisposição a neoplasias; devem ser pesquisadas em casos de ambigüidade genital associada a IRC e(ou) TW; de amenorréia primária com IRC; e aniridia, ambigüidade genital e dismorfismos. (**Arq Bras Endocrinol Metab 2008; 52/8:1236-1243)**

Descritores: Diferenciação sexual; Gene *WT1*; Síndrome de Denys-Drash; Síndrome de Frasier; Síndrome WAGR

INTRODUCTION

Sex determination is a complex and yet not fully elucidated process which depends on a complex network of interrelated genes. Gonadal development starts by the end of the 5th week of gestation with the migration of primordial germ cells from the yolk sac to the gonadal anlage. Formation of the primordial gonads, which have no apparent sexual differences up to 8 weeks, depend on the expression of many genes, including *SF-1* (steroidogenic factor 1) (1), *DAX1* (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) (2) and *WT1* (*Wilms tumour 1*) (3).

WT1 (OMIM 607102) is located at 11p13 and encodes a zinc finger motif-containing transcription factor involved in regulation of growth and differentiation (4). Beyond its role in the genesis of Wilms tumour (5-6), it regulates early gonad and kidney development (7).

Alternative splicing generates four major *WT1* isoforms: an alternative splice site in intron 9 allows the addition of three amino acids (KTS) between zinc fingers 3 and 4 and the fifth exon, encoding 17 aminoacids, may or may not be present. These isoforms are highly conserved among different species and play a crucial role in normal gene function. Gene action depends on the predominant isoform: WT1 (-KTS) variants act as transcriptional regulators, while *WT1* (+KTS) participates on the regulation of certain genes at the post-transcriptional level (8-10).

WT1 (-KTS) isoforms act in association with the product of *SF-1* to promote expression of anti-müllerian hormone (AMH), responsible for regression of the müllerian ducts in male embryos. The product of DAX1 can repress the synergistic action of *WT1* and SF-1, resulting in down-regulation of AMH (11). In vitro experiments suggested that *WT1* (-KTS) variants are also responsible for transcriptional activation of *SRY*, which activates the male differentiation pathway (10).

Mutations in *WT1* are found in a variety of syndromes, including Denys-Drash (DDS, OMIM 194080), Frasier (FS, OMIM 136680) and WAGR (OMIM 194072) syndromes. Both DDS and FS are characterized by gonadal and renal anomalies and predisposition to neoplasia associated with "de novo" constitutional *WT1* point mutations with a negative dominant effect (12).

In DDS, mutations often occur in the zinc finger region abolishing DNA binding capacity and leading to sex ambiguity as a result of dysgenetic testis, diffuse mesangial sclerosis with chronic renal disease and high incidence of Wilms tumour (WT) (13-14). The clinical picture of FS includes dysgenetic gonads with male-to-female sex reversal in 46,XY subjects and pubertal delay in both sexes, nephrotic syndrome and focal segmental glomerulosclerosis leading to chronic renal disease , and high incidence of gonadoblastoma but not of WT. Mutations in FS affect the splice site in intron 9, with *WT1*(+KTS) isoforms (15-16) losses.

WAGR (WT, aniridia, genitourinary malformations, mental retardation) is a contiguous gene syndrome arising from deletions of chromosome 11p13 which encompass at least both the *PAX6* and *WT1* genes (17-19).

We report five patients followed in a reference service for disorders of sex development which illustrate the broad spectrum of presentation of WT1-associated disorders.

SUBJECTS AND METHODS

Patients

The five patients were evaluated by the Interdisciplinary Study Group of Disorders of Sex Development (GIEDDS) of the State University of Campinas, São Paulo. The protocol was approved by the local Ethics Committee (N. 434/06) and informed consent was obtained from the parents of the children included in the study.

Laboratory assays

LH, FSH, testosterone were measured by electrochemiluminescence (BM/Hitachi Elecsys 2010, Roche Diagnostics, Boehringer, Mannheim, Germany).

Karyotype

Chromosome analysis of peripheral blood lymphocytes was performed by G-banding at 500-600 bands resolution using standard procedures.

Genomic DNA extraction, amplification and sequencing

Genomic DNA from peripheral blood leukocytes was purified by Proteinase K digestion and phenol/chloroform extraction followed by ethanol precipitation using standard techniques (20).

The 10 exons and the exon-intron junction regions of the *WT1* gene were PCR amplified from genomic DNA with primers described in Table 1. Genomic sequence of the *WT1* gene was obtained in the published sequence ENSG00000184937 (www.ensembl.org).

The final volume of all reactions was 50 uL and contained 10X *Taq* DNA polymerase buffer (Invitrogen, CA, USA), 1.0-1.5 mM MgCl2, 2 mM of each dNTP, 20 pmol of each primer, 300-500 ng genomic DNA templates 2 units recombinant *Taq* DNA polymerase (Invitrogen), 5% DMSO used only for exon 1. After a first denaturation step (5 min, 94°C), the cycling profile was: 94°C, 1 min; 53,5°C – 63,5°C, 1 min; 72°C 1-6 min (30 cylcles), followed by 5 min at 72°C (final extension). The size of the PCR products was verified in 1% agarose gel electrophoresis stained with ethidium bromide. Before sequencing, purification of PCR products was performed using the Wizard® SV Gel and PCR clean-up system (Promega, Madison, WI, USA). Further direct sequencing using ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Kit (ABI PRISM/PE Biosystems, Foster City, CA, USA) was carried out in two separate reactions for each exon, except for exon 1 which required four reactions, using sense and antisense primers (Table 1). The sequences were obtained in an automatic sequencer ABI PRISM 3700 DNA Analyzer (ABI PRISM/PE Biosystems). Free softwares Chromas Lite and CLC Sequence Viewer v.5.0.1 were used to analyze and compare sequences with the published *WT1* sequence at Ensembl database.

RESULTS

Case 1

A 1-month-old child was referred to us due to sex ambiguity. He was born at term after an uneventful preg-

Table 1. *Primers* designed for *WT1* coding sequence amplification.

Primer	Direction	Sequences 5'-3'	nt [2]	Ta (°C)[3]	Size (bp)[4]
Exon 1	Forward	TGAGTGAATGGAGCGGCCGAG	512-532	60.5	1049
	Reverse	TTGGGAAGCAGCTGGGTAAGAG	1539-1560		
Intron 1Int [1]	Forward	TTCATCAAACAGGAGCCGAG	1211-1230		
Intorn 1Int[1]	Reverse	AAAGTGGACAGTGAAGGCGC	1269-1288		
Exon 2-3	Forward	CTGTCCCAAGGTCACATCCAG	7324-7344	57.5	1015
	Reverse	AAGTAGTAGAGTGGAGTCGAGGC	8313-8338		
Intorn 2Int[1]	Reverse	ATTTGCTGTGGGTTAGGAATTC	7710-7731		
Intron 2Int[1]	Forward	GGCTTAGCTTCTTGCATTCTG	7921-7941		
Exon 4-5	Forward	GATTTGCATATTCTGTCATTCTG	18351-18373	53.4	1406
	Reverse	ATGCTACCCTGATTACCCACG	19737-19757		
Intron 4Int[1]	Reverse	AAGCGTTCTAATGTCACAGAGAG	18678-18700		
Intron 4Int[1]	Forward	GCACTCTTGATAGCTAGCTTGATG	19475-19498		
Exon 6	Forward	TGCATCTAAAGTGGCCCCATG	35945-35965	57.5	375
	Reverse	AAAGGAGCCTGCAGTGAAGAAG	36298-36319		
Exon 7	Forward	TGGGGATCTGGAGTGTGAATG	39586-39606	56.6	442
	Reverse	TCTTTACAACACCTGGATCAGACC	40004-40027		
Exon 8-9	Forward	TACCCTAACAAGCTCCAGCG	43258-43277	55,1	1037
	Reverse	TCTCTCAACTGAGTCTAAACCTTAG	44271-44295		
Intorn 8Int[1]	Reverse	GAGAATCATGAAATCAACCCTAG	43522-43544		
Intron 8Int[1]	Forward	TGAGGCAGATGCAGACATTG	43949-43968		
Exon 10	Forward	CGGGCCTTGATAGTTGAACTTG	46892-46913	56.1	890
	Reverse	GTTTCTTAAGAGCAGTGTGCCAG	47759-47781		

[1]Internal primers used only for sequencing; [2]nucleotide position in the sequence ENSG00000184937; [3]Anealing temperature used in PCR; [4]size of amplified fragments.

nancy with a birth weight of 3,655 g and length 50 cm. He was the second child of unrelated parents and family history was unremarkable. He had a 2-cm phallus with *chordee*, penoscrotal hypospadias, shawl scrotum, bilateral cryptorchidism, and there was no dysmorphic picture associated to sex ambiguity. Sonography revealed no mullerian derivatives while genitography showed a urogenital sinus.

His karyotype was 46,XY, and there were normal basal levels of LH (5.4 U/L), FSH (3.8 U/L), free (5.4 pg/mL) and total (157 ng/dL) testosterone (T). When the child was 6 months old, a hCG stimulation test was performed (three intramuscular injections of Chorionic Gonadotropin (Profasi®, 1,000 IU, on successive days), and testosterone levels increased from 157 to 395 ng/dL. He developed unilateral WT and chronic renal disease at 8 months, and died 2 months later as a result of septicemia.

An 1186G>A heterozygous mutation was detected in exon 9 and confirmed the diagnosis of DDS; this case was first reported by Tagliarini and cols. (21).

Case 2

A 17.3-year-old girl was evaluated for primary amenorrhea and hypergonadotrophic hypogonadism. She was born at term after an uneventful pregnancy, with a birth weight of 3,560g and length 46cm. She was the second child of unrelated parents, and family history was unremarkable. She was subject to renal transplantation at 11 years as a consequence of chronic renal disease; at the same age, bilateral inguinal hernia repair was performed.

She referred spontaneous pubertal onset. On physical examination, she had normal external genitalia and pubertal development was on Tanner stage B3P2. There was no dysmorphic picture. Ultrasound revealed a 2.8cm^3 uterus, and gonads could not be found.

Her karyotype was 46,XY, and there were high levels of FSH (188 U/L) and LH (46 U/L) and low estradiol (11pg/mL). Bilateral gonadectomy was performed, and histology revealed a right dysgenetic gonad with mesonephric remnants and a gonadoblastoma on the left. Female hormonal replacement therapy was initiated later on. Molecular analysis revealed an IVS9+4, C>T heterozygous mutation in intron 9 (Figure 1), thus confirming the diagnosis of Frasier syndrome.

Case 3

A 18-year-old girl was referred with a suspected diagnosis of FS to molecular analysis. She was the second child of unrelated parents, and family history was unremarkable. The girl had chronic renal disease treated with peritoneal dialysis, and primary amenorrhea was investigated when she was 15 years old – cytogenetic investigation revealed a 46,XY karyotype, bilateral gonadectomy was performed and histology revealed dysgenetic gonads. She has been on HRT since then.

On physical examination, she had no dysmorphic picture, external genitalia were normal, and breast development was incomplete.

Molecular analysis revealed the same mutation of case 2, thus confirming the diagnosis of FS.

Case 4

A 9-year-old boy presented with a history of sex ambiguity, aniridia, mental and motor retardation and dysmorphic features. He was born at term by cesarian section for breech presentation, after an uneventful pregnancy, with a birth weight of 2,550g. He was the

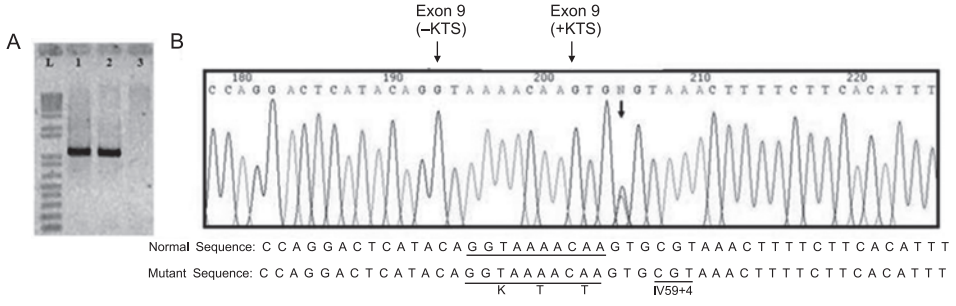

Figure 1. A) The 1037-bp exon 8-9 fragment was amplified by polymerase chain reaction (PCR). L- 1 kb-Plus Ladder (Invitrogen); patient (1), normal control (2), control without DNA (3); B) Eletropherogram of exon 9 sequence showing the C>T heterozygous mutation on *WT1* intron 9. The KTS motif and the positions of alternative splicings are denoted.

first child in a sibship of three; his parents were not related, and family history was unremarkable.

Physical examination revealed flat occiput, small and dysmorphic ears, short upslanted palpebral fissures, aniridia, nystagmus, short nose with high nasal bridge, clinodactyly of the V fingers, bilateral single transverse palmar crease, predominance of arches on the fingertips, fusiform fingers, nail hypoplasia, increased intermamillary distance and diastasis recti. He had a 4.5cm-phallus, bifid scrotum, penoscrotal hypospadia and nonpalpable gonads.

Ophthalmologic evaluation revealed macular hypoplasia. The testes were not seen on pelvic ultrasound and genitography did not reveal a urogenital sinus.

He had prepubertal levels of LH (<0.2 U/L), FSH (0.4 U/L), total (<20 ng/dL) and free (1 pg/mL) testosterone. An hCG stimulation test was performed, and testosterone levels increased from <20 to 396 ng/dL. Cytogenetic investigation revealed a *de novo* 46,XY,del(11p) karyotype, thus leading to the diagnosis of WAGR syndrome.

Case 5

A 3-month-old boy was evaluated for aniridia and dysmorphic features. He was born in the 38th week of gestation by cesarian section, and intrauterine growth retardation was noted at the 7th month. Birth weight was 2,650g and length 44cm. He was the only child of unrelated parents, and family history was unremarkable.

On physical examination, he presented high forehead, low anterior hairline, dysmorphic ears, anteverted nostrils, notched *alae nasi*, long and flat philtrum, thin upper lip, retrognathism, short neck, single transverse palmar crease and hypoplastic nails. He had a 3.5-cm phallus, bilateral cryptorchidism and hypoplastic scrotum. Ophthalmologic evaluation revealed photophobia, nystagmus, remnants of pupillary membrane and peripheral iris and mottled retinal pigment epithelium.

He had normal levels of LH (9.5 U/L), FSH (8.8 U/L), and total testosterone (669 ng/dL) for age. His karyotype was 46,XY,del (11p) *de novo* (Figure 2), leading to the diagnosis of WAGR syndrome. When he was 8 months old, a unilateral Wilms tumour was detected by sonography. He was subject to nephrectomy, chemotherapy and radiotherapy. There was no tumor relapse until the age of 4 years, and renal function remained normal. Data on these five cases are summarized in Table 2.

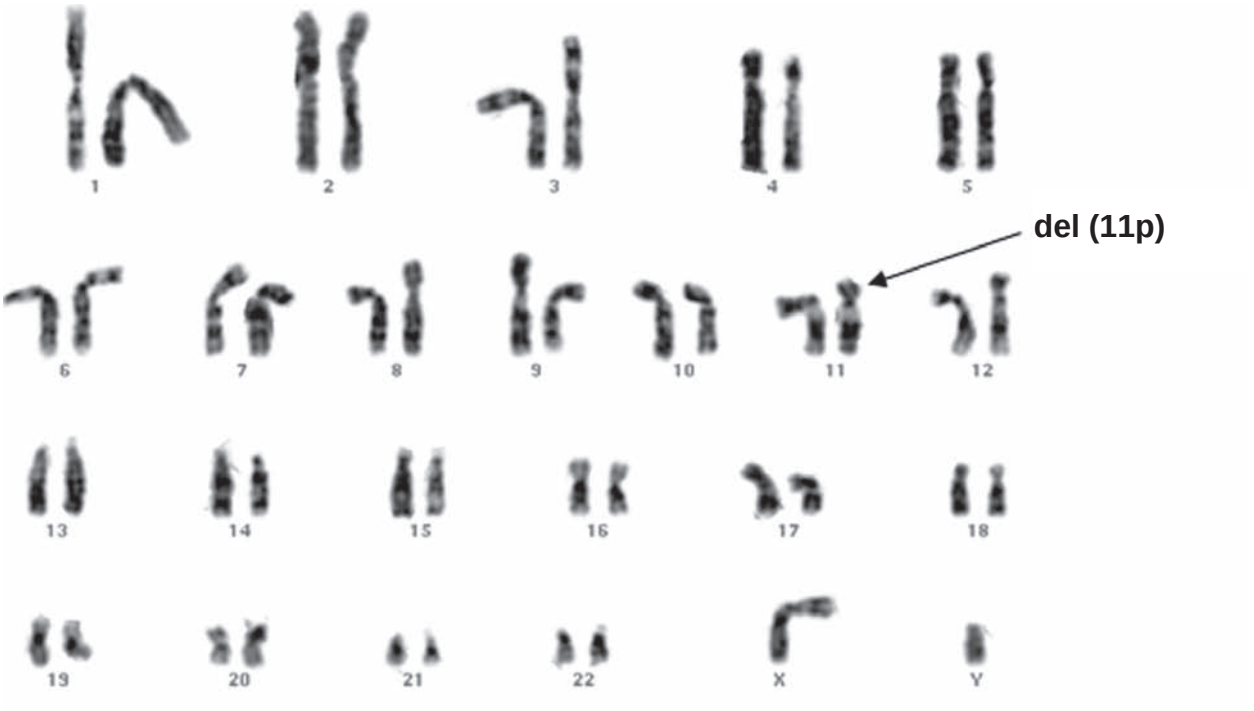

Figure 2. Karyotype of patient 5.

Table 2. Description of five 46,XY patients with WT1-related disorders.

N	Age at diagnosis	Sex assignment	Clinical picture	Neoplasia	*WT1*	Diagnosis
1	1 mo	M	penoscrotal hypospadias, BL cryptorchidism, ESRD*	Wilms tumour	1186G>A	Denys-Drash syndrome
2	17 y	F	Primary amenorrhea, hypergonadotrophic hypogonadism, ESRD	L gonado-blastoma	IVS9+4, C>T	Frasier syndrome
3	18 y	F	Primary amenorrhea, hypergonadotrophic hypogonadism, ESRD	–	IVS9+4, C>T	Frasier syndrome
4	9 y	M	Aniridia, perineal hypospadia, BL cryptorchidism, dysmorphic picture, motor and speech delay, mental deficiency	–	11p13 deletion	WAGR
5	2 mo	M	Aniridia, BL cryptorchidism, dysmorphic picture, motor and speech delay	Wilms tumour	11p13 deletion	WAGR

BL = bilateral; ESRD = end stage renal disease; F = female; L = left; M = male; NB = newborn; *deceased (10 months)

DISCUSSION

Disorders of gonadal development (DGD) are a highly heterogeneous group of disorders of sex development (DSD) and include individuals with dysgenetic gonads (streaks), dysgenetic or rudimentary testes and true hermaphroditism or ovotesticular DSD. Some 46,XY individuals with DGD are born with sex ambiguity, and thus may be evaluated in infancy. However, those with female internal and external genitalia (male-to-female sex reversal) may be diagnosed only in adolescence because of pubertal delay. The latter are of great concern because of the risk of neoplastic transformation of dysgenetic gonads, which is significantly elevated after adolescence (22). Hormonal activity of gonadoblastoma may be found in some patients (23); in case 2, for instance, there was spontaneous breast development which may be due to an estrogen-producing gonadoblastoma.

Among DGD, WT1-related disorders are characterized by the association of gonadal and renal anomalies. As a consequence, screening for mutations in *WT1* should be considered in 46,XY patients with ambiguous genitalia associated with chronic renal disease and (or) WT and in 46,XY females with hypergonadotrophic hypogonadism and history of chronic renal disease, thus allowing the diagnosis of DDS and FS.

In addition, all newborns with aniridia who do not have a family history of this ocular anomaly must be subject to high-resolution cytogenetic testing, which detects deletions involving 11p13 in up to 20% of individuals (24). FISH testing with probes spanning *PAX6*, *WT1*, the regions flanking *PAX6*, and the intervening sequence between *PAX6* and *WT1* can also be used to detect cryptic deletions in individuals with other clinical features of WAGR and normal cytogenetic studies (24).

Genotype-phenotype correlations in WT1-related disorders are well established. Mutations in DDS patients inactivate DNA binding by the zinc fingers, leading to early and severe impairment of renal function, dysgenetic testes and high incidence of WT, while in FS mutations in the donor splice site of intron 9 of the *WT1* gene lead typically to dysgenetic gonads, end-stage renal failure in the second decade and gonadoblastoma. In turn, the reduced haploinsufficiency of *WT1* in 11p13 deletion has a less pronounced effect on development, especially on that of the renal system.

However, there are some reports of atypical presentations, including a 46,XY child with sex ambiguity, nephrotic syndrome, gonadal tumour and normal testosterone production despite high levels of gonadotropins, who had a mutation associated with FS (25), In another study, a 46,XY child with sex ambiguity, normal testosterone production, aortic coarctation and no renal disease was found to have a P181S mutation in *WT1* inherited from the mother (26).

Diagnosis of WT1-related disorders is more difficult in 46,XX subjects, who lack features of sex ambiguity and sex reversal. However, clinicians must have in mind that WT1 mutations may be found in up to 5% of

cases of steroid-resistant nephrotic syndrome (SRNS) (27-28). Routine evaluation of patients with this syndrome would allow early diagnosis of both DDS and FS in both sexes.

The heterozygous 1186G>A (D396N) mutation in exon 9 of patient 1 leads to an aspartic acid-asparagine substitution changing the structural organization of the third zinc finger of the WT1 protein. It was described in 1991 (13) and is a frequent finding in DDS; the most frequent mutation is 1180C>T (R394W) (39.6%) (29). The apparent dominant-negative nature of DDS mutations results from the action of altered WT1 in blocking the normal activity of the wildtype protein (12).

The heterozygous mutation in intron 9 found in both patients with FS (IVS9+4, C>T) is the most frequent mutation identified in these patients (52%) (15,25). This and the other four different mutations described in intron 9 of *WT1* in patients with FS lead to reversal of the (+KTS)/(-KTS) ratio from 2:1 to 1:2 (15-16). Most of the patients with FS show the +4 C>T and +5G>A mutations; this hotspot is probably a consequence of the potential to deaminate 5-methylcytosine at the +4/+5 CpG dinucleotide (16).

Recurrence risk of WT1-related disorders varies according to each specific situation. DDS and FS usually arise as a consequence of *de novo* mutations, while 11p deletion in WAGR syndrome may be *de novo* or may result from transmission by a parent with a balanced chromosome rearrangement (24).

In conclusion, constitutional abnormalities of WT1 should be considered in patients with ambiguous genitalia and renal disease (chronic renal disease or Wilms tumors), primary amenorrhea with chronic renal disease, and those with aniridia, genital ambiguity and dysmorphic picture with or without WT.

Acknowledments: We are grateful to the Main Clinical Laboratory of the University Hospital and to the Cytogenetics Laboratory of the Department of Medical Genetics of State University of Campinas (Unicamp). No potencial conflict of interest relevant to this article was reported.

REFERENCES

1. Luo X, Ikeda, Y, Parker, KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell. 1994;77:481-90.
2. Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G. Mouse Dax1 expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. Nature Genet. 1996;12:404-9.
3. Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, et al. The candidate Wilms' tumour gene is involved in genitourinary development. Nature. 1990;346: 194-7.
4. Rose EA. Glaser T, Jones C, Smith CL, Lewis WH, Call, KM, et al. Complete physical map of the WAGR region of 11p13 localizes a candidate Wilms' tumour gene. Cell. 1990;60:405-508.
5. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumour locus. Cell. 1990;60:509-20.
6. Gessler M, Konig A, Bruns GAP. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. Nature. 1990;343:774-8.
7. Pritchard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gosden C, et al. The candidate Wilms' tumour gene is involved in genitourinary development. Nature. 1990;346: 194-7.
8. Haber DA, Sohn RL, Buckler AJ, Pelletier J, Call KM, Housman DE. Alternative splicing and genomic structure of the Wilms tumour gene WT1. Proc Nat Acad Sci. 1991;88:9618-22.
9. Laity JH, Chung J, Dyson HJ, Wright PE. Alternative splicing of Wilms' tumour suppressor protein modulates DNA binding activity through isoform-specific DNA-induced conformational changes. Biochemistry. 2000;39:5341-8.
10. Hossain A, Saunders GF. The human sex-determining gene SRY is a direct target of WT1. J Biol Chem. 2001;276:16817-23.
11. Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA. Wilms' tumour 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. Cell. 1998;93:445-54.
12. Moffett P, Bruening W, Nakagama H, Bardeesy N, Housman D, Housman DE, Pelletier J. Antagonism of WT1 activity by protein self-association. Proc Natl Acad Sci USA. 1995;92:11105-9.
13. Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, et al. Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. Cell. 1991;67(2):437-47.
14. Patek CE, Little MH, Fleming S, Miles C, Charlieu JP, Clarke AR, et al. A zinc finger truncation of murine WT1 results in the characteristic urogenital abnormalities of Denys-Drash syndrome. Proc Natl Acad Sci USA. 1999;96(6):2931-6.
15. Barbaux S, Niaudet P, Gubler M-C, Grunfeld J-P, Jaubert F, Kuttenn F, et al. Donor splice-site mutations in WT1 are responsible for Frasier syndrome. Nature Genet. 1997;17:467-70.
16. Klamt B, Koziell A, Poulat F, Wieacker P, Scambler P, Berta P, Gessler M. Frasier syndrome is caused by defective alternative splicing of WT1 leading to an altered ratio of WT1 +/-KTS splice isoforms. Hum Mol Genet. 1998;7(4):709-14.
17. Miller RW, Fraumeni Jr. JF, Manning MD. Association of Wilms' tumour with aniridia, hemihypertrophy and other congenital malformations. New Eng J Med. 1964;270:922-7.
18. Schmickel RD. Chromosomal deletions and enzyme deficiencies. J Pediat. 1986;108:244-6.
19. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GAP. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. Nature. 1990;343: 774-8.

20. Sambrook J, Fritsch EF, Maniatis TE. Molecular cloning, a laboratory manual. Cold Spring Harbor, New York. 1989.
21. Tagliarini EB, Assumpção JG, Scolfaro MR, Mello MP, Maciel-Guerra AT, Guerra Júnior G, et al. Mutations in SRY and WT1 genes required for gonadal development are not responsible for XY partial gonadal dysgenesis. Braz J Med Biol Res. 2005;38(1):17-25.
22. Verp MS, Simpson JL. Abnormal sexual differentiation and neoplasia. Cancer Genet Cytogenet. 1987;25:191-218.
23. Hoepffner W, Horn LC, Simon E, Sauerbrei G, Schröder H, Thamm-Mücke B, et al. Gonadoblastoma in 5 patients with 46,XY gonadal dysgenesis. Exp Clin Endocrinol Diabetes. 2005;113(4):231-5.
24. Hingorani M, Moore A. Aniridia. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database on the internet). Seattle: University of Washington, 2002 [Updated 2008 July 12; cited 2008 August 19]. Available from: http://www.genetests.org.
25. Melo KF, Martin RM, Costa EM, Carvalho FM, Jorge AA, Arnhold IJ, et al. An unusual phenotype of Frasier syndrome due to IVS9 +4C>T mutation in the WT1 gene: predominantly male ambiguous genitalia and absence of gonadal dysgenesis. J Clin Endocrinol Metab. 2002;87(6):2500-5.
26. Köhler B, Pienkowski C, Audran F, Delsol M, Tauber M, Paris F, et al. An N-terminal WT1 mutation (P181S) in an XY patient with ambiguous genitalia, normal testosterone production, absence of kidney disease and associated heart defect: enlarging the phenotypic spectrum of WT1 defects. Eur J Endocrinol. 2004;150(6):825-30.
27. Cho HY, Lee JH, Choi HJ, Lee BH, Ha IS, Choir Y, et al. WT1 and NPHS2 mutations in Korean children with steroid-resistant nephrotic syndrome. Pediatr Nephrol. 2008;23(1):63-70.
28. Gbadegesin R, Hinkes B, Vlangos C, Mucha B, Liu J, Hopcian J, et al. Mutational analysis of NPHS2 and WT1 in frequently relapsing and steroid-dependent nephrotic syndrome. Pediatr Nephrol. 2007;22(4):509-13.
29. Little M, Wells C. A clinical overview of WT1 gene mutations. Hum Mutat. 1997;9(3):209-25.

Correspondence to:

Andréa Trevas Maciel Guerra
Department of Medical Genetics, Faculty of Medical Sciences, PO Box 6111, Unicamp
13083-970, Campinas, SP, Brazil
E-mail: atmg@fcm.unicamp.br