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Original Article

Gonadal Function in 15 Patients Associated with WT1 Gene Mutations

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Abstract. Denys-Drash syndrome (DDS) and Frasier syndrome (FS) are caused by mutations of the WT1 gene. These disorders are characterized by renal disease, abnormality of male sex differentiation, and Wilms' tumor and gonadoblastoma. There have been few reports on gonadal function in a large series of patients with mutations of the WT1 gene. Here, we evaluated the relation between gonadal function and the phenotype of external genitalia in 15 Japanese patients with WT1 mutations. We confirmed three sets of information. First, if a diagnosis of DDS and FS is arrived at by genetic analysis, there are some overlaps in the phenotypes of external genitalia and renal complications. Second, the responses of serum T for the human CG (HCG) loading test coincided with the phenotype of external genitalia in both DDS and FS, except two patients. One DDS patient had male type external genitalia with a low level of serum T response, and one FS patient had complete female external genitalia despite a definite serum T response to HCG stimulation. Third, four FS patients had incomplete development of pubic hair, together with low DHEA-S levels.

Key words: Drash syndrome, Frasier syndrome, Wilms' tumor-1 gene

Introduction

Heterozygous mutations in the WT1 gene, located on the short arm of chromosome 11 and encoding a zinc-finger transcription factor involved in renal and gonadal development, have been reported in most patients with Denys-Drash syndrome (DDS) and Frasier syndrome (FS) (1, 2). The mutations of DDS generally reside in the DNA binding domain, and FS is caused by the splicing donor site of intron 9.

Clinical diagnose of DDS and FS have usually been made based on phenotypes of patients. A typical phenotype of DDS patients with 46,XY consists of ambiguous genitalia, high risk of Wilms' tumor, and renal failure within the first few years of life, whereas that in FS patients with 46,XY is female type external genitalia, high risk of gonadoblastoma, and development of renal

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Table 1 Clinical features of 46, XY DDS patients

Patient no	WT-1 mutation	Gonad	External & internal genitalia	Renal disease	Age at dialysis	Wilms' tumor
D1	394 Arg→Trp	Streak gonad	Complete female type, Müllerian ducts remnants	DMS	1 yr	+
D2	385 Cys→Arg	Streak gonad	Complete female type, Müllerian ducts remnants	FSGS	2 yr	–
D3	342 Met→Arg	Dysplastic testis	Complete female type, Müllerian ducts remnants	ESRD	1 yr	–
D4	394 Arg→Trp	Hypoplastic testis	Cryptorchism, penile type hypospadia with enlarged prostate utricle	DMS	1 yr	–
D5	394 Arg→Trp	Hypoplastic testis	Cryptorchism, penile type hypospadia with enlarged prostate utricle	DMS	1 yr	–
D6	396 Asp→Asn	NA	Cryptorchism, penile type hypospadia with enlarged prostate utricle	DMS	1 yr	–
D7	364 Phe→Ser	NA	Cryptorchism without enlarged prostate utricle	FSGS	6 yr, proteinuria without renal failure	–
D8	312 Arg→Gln	NA	Complete male type without enlarged prostate utricle	ESRD	2 yr	–

DMS; Diffuse mesangial sclerosis, FSGS; Focal and segmental glomerular sclerosis, ESRD; endstage renal disease, NA; not available.

failure in the second decade of life. Gonadal function of 46, XY DDS patient has been reported to show a broad spectrum and 46, XY FS patients typically show no gonadal function. Gonadal function in 46,XX patients with either DDS or FS has been reported to be almost normal (3, 4).

To our knowledge, hormonal evaluation of gonadal function in large series of patients with mutations of the WT1 gene are rare. Thus, we studied gonadal function and its relation with genital phenotype in 15 Japanese patients with WT1 mutations.

Subjects

The 15 Japanese patients were followed up at Tokyo Metropolitan Kiyose Children's Hospital from1970 to 2003 and were clinically selected by both phenotypes of external genitalia and renal complication. Their diagnose were confirmed by genetic analysis of the WT-1 gene as previously reported (5, 6). There were eight DDS patients with 46,XY (Table 1), six FS patients with 46,XY and one FS patient with 46,XX (Table 2). All eight patients with DDS had heterozygous missense mutations at exon 7–9 of the WT1 gene. All of the patients with FS had heterozygous

Table 2 Clinical features of 46, XY FS patients (F1–F6) and 46, XX FS patient (F7)

Patient no	WT-1 mutation	Gonad	External & internal genitalia	Renal disease	Age at dialysis	Gonado-blastoma
F1*	Intron 9 4 c→t	NA	Complete female type, Müllerian ducts remnants	Dysplasia	0.6 yr	NA
F2*	Intron 9 4 c→t	NA	Complete female type, Müllerian ducts remnants	Dysplasia	0.6 yr	NA
F3**	Intron 9 4 c→t	Streak gonad	Complete female type, Müllerian ducts remnants	FSGS	22 yr	–
F4**	Intron 9 4 c→t	Streak gonad	Complete female type, Müllerian ducts remnants	FSGS	24 yr	–
F5	Intron 9 2 t→c	Streak gonad	Female type, clitoromegaly Müllerian ducts remnants	FSGS	23 yr	–
F6	Intron 9 5 g→a	Streak gonad	Complete female type, Müllerian ducts remnants	FSGS	12 yr	–
F7 46, XX	Intron 9 4 c→t	Ovary	Complete female type, menarche at 12 yr, regular menstrual cycle	DMS	4 yr	NA

DMS; Diffuse mesangial sclerosis, FSGS; Focal and segmental glomerular sclerosis, NA; not available. Patient F1* and F2*, and patient F3** and F4** were monozygotic twins.

mutations in the splicing-donor-site of intron 9 +2–5 of the WT1 gene. The main complaint of DDS patients was persistent proteinuria and that of FS patients was absence of secondary sexual development in addition to proteinuria. At present, the DDS patients are 4.0 to 26 yr old (median 10.4 yr), and the FS patients are 22 to 34 yr old (median 25.3 yr). Both DDS and FS patients did not receive steroid therapy before renal transplantation.

Methods

The LH releasing-hormone (LHRH) loading test was performed with a dose of 100 μg/m^2, intravenously. Serum gonadotropins were measured by immunoradiometric assay, except those of patient 3 with DDS, whose gonadotropins were measured by polyclonal radioimmunoassay. The intra-assay coefficient of variation (CV) and inter-assay CV for LH (5–6 mIU/ml) were 4.4 (n=10) and 5.3% (n=5), respectively. The intra-assay CV and inter-assay CV for FSH (3–4 mIU/ml) were 3.6 (n=10) and 3.5% (n=5), respectively. The lower limits of sensitivity of the standard curves were 0.1 mIU/ml for both LH and FSH. The ranges in normal serum peak values in males following the LHRH loading test have not been studied in detail. However, our reference LH and FSH levels in prepubertal males are about 2–4 mIU/ml and 2–10 mIU/ml, respectively (unpublished). The human CG (HCG) loading test was performed with a dose of 4000 U/m^2/day, intramuscularly for three days (7). Serum testosterone was measured by radioimmunoassay. The intra-assay CV and inter-assay CV for T (300–400 ng/dl) were 6.1% (n=20) and 8.3% (n=10), respectively. The lower limit of sensitivity of the standard curve for T was 5.0 ng/dl. The lower cut-off point of serum T response to HCG stimulation is 200 ng/dl (7). Serum dehydroepiandrosterone-sulfate (DHEA-S) was

Table 3 Endocrinological data of 46, XY DDS patients

Patient no	Age at LHRH loading test (yr)	LH (mIU/ml)	FSH (mIU/ml)	Age at HCG loading test (yr)	T (ng/dl)
D1	6.3	0.5→5.1	2.5→21.4	6.3	50→73
D2	NA	Basal 49.2 (19 yr)	Basal 97.1 (19 yr)	NA	Basal 20 (19 yr)
D3	NA	Basal 6.6* (8 yr)	Basal 150<* (8 yr)	NA	NA
D4	2.6	0.6→3.0	0.8→3.6	2.6	10>→340
D5	NA	NA	NA	1.0	5>→10
D6	5.5	0.5>→2.1	1.3→13.2	5.5	5>→130
D7	2.8	0.5>→2.8	2.1→7.0	2.8	10>→560
	5.6	0.5>→1.5	1.4→6.1	5.6	10>→280
D8	2.8	0.3→3.1	0.9→4.6	NA	Basal 5> (8 yr)

NA; not available. *Normal prepubertal serum levels in males (mean ± SD) measured by polyclonal RIA; LH (mIU/ml) 3.9 ± 2.6, FSH (mIU/ml) 1.5 ± 0.7 (Ref.19).

measured by radioimmunoassay. The intra-assay CV and inter-assay CV for DHEA-S (100–200 ng/ml) were 7.2% (n=20) and 4.9% (n=10), respectively. The lower limit of sensitivity of the standard curve for DHEA-S was 20 ng/ml. The range of normal serum basal values in serum DHEA-S for persons aged 14 to 16 yr is 424–4769 ng/ml (8).

Results

External and internal genitalia, gonad, and hormonal evaluation in patients with DDS (Tables 1 and 3)

DDS (D) showed a broad spectrum of phenotype of external genitalia as previously reported (1, 2). Three patients (D1–D3) showed complete female type genitalia. Three patients (D4–D6) showed an ambiguous type with hypospadia. Two patients (D7, D8) had complete male type genitalia.

Regarding a genotype-phenotype correlation, three patients (patients D1, D4, and D5) had an identical missense mutation of the WT1 gene, however their phenotypes of external genitalia were different. The phenotype of external genitalia in patient D1 was complete female type, however, those of patients D4 and D5 were male type with bilateral undescended testes and hypospadia.

Among the three patients with complete female type external genitalia (patients D1–D3), patients D1 and D2 had streak gonads. In patient D3, streak gonad and gonadoblastoma were found at the age of eight years. All of them had residual Müllerian duct with uterus and upper portion of vagina. Among the three patients with bilateral undescended testes and hypospadia (patients D4–D6), patients D4 and D5 had hypoplastic testes and an enlarged prostate utricle. Patient D7 with male type external genitalia had bilateral undescended testes. Patient D8 had isolated DMS, as was reported previously (5).

HCG loading tests were performed on five patients among eight patients with DDS when they were under 7 yr old. LHRH loading tests were performed on five patients with DDS when they were under 7 yr of age. Among the patients with complete female type external genitalia (patients D1–D3), patient D1 had no serum T increments in the HCG loading test. Patient D2 had high basal levels of LH and FSH when she was 19 yr old, suggesting that she had primary hypogonadism. Among patients with bilateral

Table 4 Endocrinological data of 46, XY FS patients (F1–F6) and 46, XX FS patient (F7)

Patient no	Age at LHRH loading test (yr)	LH (mIU/ml)	FSH (mIU/ml)	Age at HCG loading test (yr)	Testosterone (ng/dl)	DHEA-S (ng/ml)
F1*	NA	Basal 81 (18 yr)	Basal 226	18	43→47	NA
F2*	NA	Basal 116 (18 yr)	Basal 321	18	200→235	NA
F3**	13	71→268	87→138	13	20→20	58 (15 yr)
F4**	13	45→210	68→125	13	20→10	97 (15 yr)
F5	NA	Basal 19 (21 yr)	Basal 35 (21 yr)	19	60→130	204 (21 yr)
F6	16	100→525	252→408	16	10>→30	NA
F7 46, XX	NA	NA	NA	NA	E_2 100 pg/ml (14 yr)	

NA; not available. Patient F1* and F2*, and patient F3** and F4** were monozygotic twins.

undescended testes and hypospadia (patients D4–D6), their responses to the HCG loading test were variable: a normal response in patient D4, no response in patient D5, and a low response in patient D6. In patients D4 and D6, their serum LH and FSH responses to LHRH were normal prepubertal levels. In patient D7, the peak level of serum T response to the HCG loading test at the age of 5.6 yr was lower than that at the age of 2.8 yr. The results of serum LH and FSH responses to the LHRH loading test in patients D7 and D8 were within the normal prepubertal ranges.

External and internal genitalia, gonad, and hormonal evaluation in patients with FS (Tables 2 and 4)

All six FS (F) patients with 46,XY had female external genitalia and residual Müllerian duct with uterus and upper portion of vagina. Patient F5 had moderate clitoromegaly and received clitoroplasty.

Four FS patients with 46,XY (patients F3–F6) received gonadectomies, and their histology revealed streak gonads. F1 and F2 did not receive gonadectomies, although we explained to them risk of gonadoblastoma.

The HCG loading test was performed at over 13 yr of age in six FS patients with 46,XY (patients F1–F6). Four FS patients with 46,XY (patients F1, F3, F4, and F6) showed no response of serum T to the HCG loading test. Patient F5 with clitoromegaly had a low but definite peak level of serum T of 130 ng/dl in response to the HCG loading test. Patient F2 had a subnormal peak level of serum T of 235 ng/dl in response to the HCG loading test. Patients F1 and F2 were monozygotic twins, but they had different responses to the HCG loading test. The LHRH loading test was performed on three patients (patients F3, F4 and F6) at over 13 yr of age. Their results were consistent with hypergonadotropic hypogonadism.

At present, all FS patients are over 20 yr of age. In four patients (patients F3–F6), pubic hair appeared at the age of over 15 yr and remained at stage II–III at the age of over 20 yr. Three patients (F3–F5) out of four had low basal levels of DHEA-S before renal transplantation followed by steroid therapy.

In patient F9 with 46,XX, menarche appeared at the age of 12 yr and thereafter a regular menstrual cycle persisted, indicating the WT-1 gene does not play an important role in ovarian development and function as described previously (3, 4). She suffered from endometriosis at the age of 20 yr.

Discussion

If the diagnosis of DDS and FS is arrived at by genetic analysis, namely, mutations in the DNA binding domain for DDS and those in the splicing donor site of the intron 9 for FS, there are some overlaps in phenotypes of external genitalia and renal complications. Mild masculinization was found in the external genitalia of patient F7. It has been reported that a 46, XY FS patient had a variation of external genitalia from female to male type (9–12). Overlaps in renal complication were observed in our study. Patients F1 and F2 had renal failure at ages under three years old and patient D7 had proteinuria without renal failure at the age of 6 yr, as was reported by our renal group (the same patient in reference 5). Similar findings were also reported by other groups (13, 14).

In one DDS and one FS patients, there was a discrepancy between the phenotype of the external genitalia and the results of the HCG loading test. Patient D5 had male type genitalia, however HCG did not stimulate serum testosterone levels. Patient F2 with normal female external genitalia demonstrated a low but definite response to HCG loading test. The exact reason for these dissociations are not clear. The result of the HCG loading test during prepubertal period may not reflect testosterone secretion during the fetal age. Another explanation is that modifying gene(s) other than WT-1 could affect testosterone secretion during the fetal age, since modifications have been reported in other genes related to testosterone secretion, such as SRY and StAR (15–18).

Four 46,XY FS patients showed incomplete development of pubic hair and three patients out of these four studied had low basal levels of serum DHEA-S in our study. Melo *et al.* (12) reported also that one FS patient, caused by the WT-1 gene mutation, had poor pubic hair and that low levels of serum DHEA-S had persisted until the patient was 17 yr old. The reason for the apparent relationship between pubic hair development and low DHEA-S levels remains to be clarified.

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