Seminoma in a postmenopausal woman with a Y;15 translocation in peripheral blood lymphocytes and a t(Y;15)/45,X Turner mosaic pattern in skin fibroblasts

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Received 26 September 1997
Revised version accepted for publication 10 March 1998

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Abstract

We report an unusual case of a 55 year old Japanese woman with a seminoma but relatively normal menses. The patient was a phenotypic female with late onset menarche (18 years of age), who was amenorrhoeic for the first year, followed by menses of one to three days' slight flow with dysmenorrhoea, but an otherwise normal menstrual history. A typical seminoma was removed from the left adnexal region and an immature testis was identified separately as an associated right adnexal mass. Repeated karyotypic studies on peripheral blood lymphocyte cultures showed only 46,X,-Y,t(Y;15)(q12;p13). Cytogenetic examination of the patient's younger brother, who had fathered three healthy children, showed an identical karyotype. Mosaicism of 46,X,-Y,t(Y;15)(q12;p13)/45,X cell lines was found in skin samples from the patient's elbow and genital regions, although there were no clinical stigmata of Turner syndrome. An androgen receptor binding assay of cultured genital skin fibroblasts was negative. Molecular analysis using Southern blot hybridisation, PCR, and direct DNA sequencing showed that neither the patient nor her brother had a detectable deletion or other abnormalities of Y chromosome sequences, including the SRY (sex determining region of the Y chromosome) gene sequence. These findings suggest that Turner mosaicism of the 45,X cell line may have contributed to this atypical presentation in an XY female, although

we cannot exclude abnormalities of other genes related to sex differentiation.

(J Med Genet 1998;35:852-856)

Keywords: sex determining region of Y (SRY); seminoma; Turner mosaicism

Sexual dimorphism in mammalian embryogenesis provides a model of a developmental switch. The signal event in development of a bipotential gonad into a testis¹ is regulated in early embryogenesis by the Y chromosome.²⁻³ A regulatory gene named testis determining factor (TDF) has been mapped to the short arm of the Y chromosome (interval 1A1)⁴ by molecular genetic analysis of human sex reversal samples (XX males and XY females).⁵⁻⁸ Full sexual differentiation and formation of a functional testis must involve programming by many different genes and not simply by the isolated testis determining sequence itself. The gene SRY (sex determining region of the Y chromosome) (in mice, *Sry*) was isolated by analysis of 35 kb of DNA adjacent to the pseudoautosomal boundary in the human Y chromosome⁷ and is involved in sex differentiation.⁹ SRY gene expression is presumably triggered via upstream genes to account for its specific time and place of expression.¹⁰ Recently, Ad4BP/SF-1, WT-1 (Wilms tumour-1), DAX (dosage sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome), and SOX (SRY type HMG box) genes associated with sexual differentiation have been reported.¹¹ In addition, other genes are presumably active in the formation of the urogenital ridges even before colonisation by primordial germ cells.

Materials and methods

CASE REPORT

A 55 year old married Japanese woman without children presented to her physician with vomiting, loss of appetite, and weight loss (~10 kg over two months). The patient was 162 cm tall and weighed 48 kg; physical examination was remarkable for a firm, non-tender mass in the pelvic cavity. She had normal female external genitalia with moderate breast enlargement although pubic and axillary hair were slight. Chest x ray and intravenous pyelogram were normal.

* No children

Figure 1 Pedigree indicating child bearing history of family. The arrow indicates the proband (patient). The asterisks represent subjects without children.

Figure 2 Computed tomograms of the pelvic and abdominal cavity. (A) Tumour mass (11 × 12 cm) as a uniform water dense structure. (B) Many enlarged para-aortic lymph nodes are present at the level of the renal artery.

Past history showed that the patient did not menstruate during the year following menarche at the age of 18. Thereafter, she had regular 28–30 day menstrual cycles of one to three days of slight flow with moderately severe dysmenorrhoea. Her bleeding sometimes lasted for only one day and was scanty like staining. Menses ceased at the age of 54 after five to six years of irregular cycles. There was a family history of infertility in both men and women (fig 1).

Laboratory studies showed normal levels of AFP (alpha-fetoprotein), CEA (carcinoembryonic antigen), CA (carbohydrate antigen) 19-9, CA 125, CA 50, BFP (basic fetoprotein), and hCG (human chorionic gonadotrophin). An 11 × 12 cm water dense mass impinging on the urinary bladder was noted by computed tomography (fig 2A), along with enlargement of para-aortic lymph nodes (fig 2B).

A laparotomy was performed. The surgical specimen, submitted as a left ovarian tumour, measured 15 × 15 × 11 cm and weighed 750 g.

Figure 3 Microscopic findings of the gonads. (A) Germinoma in left gonadal tissue. A typical two cell pattern consisting of epithelial cells with a stromal lymphocytic infiltrate is present (H&E). (B) Immature seminiferous tubules observed in part of the left gonadal tumour (H&E). (C) Right gonadal tissue. Immature seminiferous tubules containing only Sertoli cells, along with stromal Leydig cell hyperplasia (H&E).

The cut surface was solid with a soft elastiform consistency and uniform light brown colour. A right adnexal mass, along with what was felt to be a rudimentary uterus and thin, short fallopian tubes, were also removed. Microscopic examination of the tumour showed findings typical of a seminoma with cords of polyhedral cells featuring clear cytoplasm separated by a prominent lymphoid infiltrate (fig 3A). Immature seminiferous tubules indicating residual testis tissue were noted in part of the tumour (fig 3B). The right adnexal mass was also diagnosed as being of testicular origin because of immature seminiferous tubules containing only Sertoli cells, along with Leydig cell hyperplasia (fig 3C) in the intervening stroma. Definitive uterine tissue could not be identified; the tissue removed as a rudimentary uterus consisted only of smooth muscle tissue. The patient subsequently underwent three courses of chemotherapy which included one course of PVB (cisplatin 70 mg/m², vinblastine sulphate 3 mg/m², bleomycin 20 mg/m²) and two courses of CAP (cisplatin 70 mg/m², adriamycin 20 mg/m², cyclophosphamide 350

Table 1 Chromosome analyses on blood, genital, and elbow skin from the patient and peripheral blood from her brother

Material	46,X,-Y,t(Y;15) (q12;p13)	45,X
Blood	100	0
Genital skin	76	24
Elbow skin	90	10
Blood (brother)	50	0

mg/m²) followed by radiation therapy (40 Gray/16 frequency/4 week) and is now felt to be in clinical remission without evidence of metastasis.

CYTOGENETIC AND MOLECULAR GENETIC EXAMINATION

Multiple chromosome analyses were conducted on peripheral blood lymphocytes and cultured fibroblasts from genital and elbow skin. The patient's father was dead, but we examined her younger brother's karyotype by routine C, G, and Q banding methods for clues

to the origin of the patient's (Y;15) translocation using CytoVision (Applied Imaging Corporation, USA). Genomic DNA was also prepared from peripheral blood leucocytes and cultured skin fibroblasts by standard techniques, and then subjected to restriction endonuclease digestion, electrophoresis, and Southern blot hybridisation using probes described below to detect the presence of various regions of the Y chromosome.¹² Two additional loci, the SRY and the proximal border of the pseudoautosomal region (PABY), were examined by polymerase chain reaction (PCR)¹³ from DNA and from paraffin embedded gonadal tissues by a modified method.¹⁴

For Southern blot studies, we used 17 Y specific fragments as probes. These detected 24 loci spanning the entire chromosome from Yp to Yq telomeric regions; the map positions of these loci have been previously defined by deletion mapping.¹⁵ The Y chromosome sequence described by Sinclair *et al*⁷ was also used as a target for PCR. Direct sequencing of the SRY region was performed using an ABI 373A fluorescent automated DNA sequencer (Applied Biosystems, USA) with Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer Corporation, USA) using custom primers from Hokkaido System Science Co Ltd, Japan. Additional sequencing was also performed using a Hitachi SQ-5500 fluorescent automated DNA sequencer (Hitachi Ltd, Japan) using primers with 5' T7 sequences following subcloning of the ~350 bp PCR product using the Takara Ligation System (Takara Shuzo Co Ltd, Japan), pT-Blue T-vector (Novagen, USA), and competent high DH5 (Toyobo, Japan) cells. Thermosequence sequencing reagents were from Amersham International plc, UK.

ANDROGEN RECEPTOR STUDIES

A genital skin sample was obtained by punch biopsy of the labia majora and cultured in 10% FCS-MEM with Eagle's salts supplemented with non-essential amino acids and antibiotics. Specific binding of 5 α -dihydrotestosterone (DHT) to androgen receptors of skin fibroblasts was measured by the method described by Brown and Imagine.¹⁶ Briefly, 10⁶ confluent cells were incubated for one hour at 37°C with 0.15-5 nmol/l [³H]DHT dissolved in MEM without fetal bovine serum. After incubation the medium was removed and an aliquot was counted to determine free radioactivity. The radioactivity remaining in the supernatant was counted by liquid scintillation spectrometry with a counting efficiency of 46% for tritium. Each 0.25 ml aliquot of the supernatant contained approximately 10-15 μ g DNA, as determined by the method of Burton.¹⁷ The maximum binding capacity (Bmax, fmol/mg DNA) and the apparent dissociation constant (Kd, 10⁻⁹ mol/l) of the DHT receptor were derived from Scatchard plots¹⁸ using linear regression analysis.

Results

Repeated chromosome studies of the patient's peripheral blood showed a 46,X,-Y,t(Y;15)(q12;p13) translocation. There was an

Figure 4 Karyotypes of the patient and her younger brother. A normal 15, a der(15), a normal 21 and 22, an X, and a der(Y) chromosome can be seen in cultured lymphocytes from the patient and her brother. The breakpoint is within band q12 of the Y chromosome and band p13 of chromosome 15. Arrows show Q and C banding positive chromosome segments in the short arm of chromosome 15. Q: Q banding, C: C banding of constitutive heterochromatin.

Table 2 Summary of DNA analyses

Probe	Locus	Patient				Brother
		Blood	Skin	R gonad	L gonad	Blood
pDP1007 47z 87-27a 52dB 52dC 87-17aB 50f2A 50f2B 50f2D 87-7a 87-4a 12f3 87-28a 87-26a,b 87-24a 87-31a 87-19a 87-6c 52dA 87-17aA 50f2E 50f2C 49f pHYIO	PABY*	+	+	+	+	+
	SRY*	+	+	+	+	+
	ZFY	+	+			+
	DXYS5	+	+			+
	DXYS73	+	+			+
	DYS27B	+	+			+
	DYS27C	+	+			+
	DYS131	+	+			+
	DYS7A	+	+			+
	DYS7B	+	+			+
	DYS7D	+	+			+
	DYS130	+	+			+
	AMGL	+	+			+
	DYS11	+	+			+
	DYS140	+	+			+
	DYS139	+	+			+
	DYS133	+	+			+
	DYS134	+	+			+
	DYS132	+	+			+
	DYS135	+	+			+
	DYF27A	+	+			+
	DYS131A	+	+			+
	DYS7E	+	+			+
	DYS7C	+	+			+
	DYS1	+	+			+
	DYZ1	+	+			+

The presence (+) or absence (–) of portions of the Y chromosome detected by Southern blot or PCR analysis (see Materials and methods for details) are indicated for samples from the patient and her brother. Loci are arranged in the order previously established on the Y chromosome.¹⁵
*Detected by PCR.

Table 3 [³H]DHT binding assay results for fibroblasts cultured from genital skin

Case	Kd (nmol/l)	Bmax (sites/cell)
C-II	1.6	4.8×10 ³
C-IV	2.5	1.1×10 ⁴
C-V	1.3	3.6×10 ³
	1.2	4.8×10 ³
	1.1	3.8×10 ³
HZ	0.9	1.1×10 ⁴
	1.2	1.4×10 ⁴
TJ-II	ND	

ND: not detected in five different experiments.
C-II, IV, and V: normal male genital skin fibroblasts; HZ: a case of partial androgen resistance.
TJ-II: the present patient's fibroblasts.

atypical Q and C banding positive chromosome segment in the short arm of chromosome 15 (fig 4). A confirmatory cytogenetic examination of the patient's younger brother, who was married and had normal children, showed the same karyotype as the patient (fig 4). Karyotypic analysis of the cultured fibroblasts from the patient's genital and elbow skin showed a minor mosaic component with a 45,X karyotype. Ratios of 45,X to 46,X,-Y,t(Y;15)(q12;p13) cells in genital and elbow skin were 24/76 and 10/90 (table 1), suggesting the possibility of heterogeneity in other body organs. Molecular probing of regions along the Y chromosome by Southern blotting, PCR, and direct sequencing approaches showed no apparent deletion of Y chromosome sequences in the patient or her brother and no mutation in the SRY gene in blood, tumour/gonadal tissue, or skin samples (table 2).
Androgen receptor binding assay in cultured genital skin showed no appreciable binding in five different experiments (table 3).

Discussion

The present case appears to be an XY female, in agreement with histological evidence of tes-

tis tissue found in both adnexae as well as the diagnosis of her tumour as a seminoma. Testicular feminisation (androgen insensitivity syndrome) was confirmed by the androgen receptor assays on cultured skin fibroblasts from the patient. It is well known that XY females with seminoma have male genitalia owing to testosterone¹ and do not have ovaries or a uterus, implying suppression of Müllerian duct development as a result of Müllerian inhibiting substance secreted by the fetal testis (Sertoli cell).¹⁹ However, unlike most XY females who present with primary amenorrhoea, our patient had a menstrual history of reasonably normal, if late onset, amenorrhoea for the first year, followed by menses of one to three days' slight flow with dysmenorrhoea.
DNA analysis showed no evidence of deletion of Y chromosome sequences including the SRY region in either the patient or her brother as a possible explanation for this patient's unusual menstrual history. Many XY females have also been reported to have no abnormality in the SRY gene.^{20 21} The presence of Leydig and Sertoli cells also provides evidence for cells dependent on the expression of Y chromosome or SRY genes.^{22 23}
We performed chromosomal analysis of the patient's genital and elbow skin to seek possible additional clues for this unusual phenotypic association. These studies showed a minor but significant mosaic population of cells with a 45,X karyotype intermixed among the majority with the constitutional 46,X,-Y,t(Y;15)(q12;p13) karyotype. Mosaicism associated with the 45,X karyotype appears to have occurred after fertilisation because of differing results among different samples, and mosaic patterns are known to depend on many factors, such as the number of blastomeres at the time of the mutational event, the cell lineage affected by the mutational event, and cell viability and selection.²⁴ We do not know if mosaicism is present in other non-ectoderm derived tissues in the present case, but it was not observed in peripheral blood lymphocytes. Unfortunately, no testis specimen was available for chromosome analysis.
The dysgenesis that characteristically produces 45,X Turner syndrome²⁵ is a common and interesting disorder. Characteristic clinical features include short stature and primary amenorrhoea, neither of which our patient exhibited. Although 45,X is the most common karyotype, this syndrome may also be caused by the partial absence of one of the X chromosomes, such as in 45,X/46,XY mosaicism.^{25 26} Turner mosaicism in gonadal and other genital tract tissues may in some modifying way have contributed to the unusual phenotypic features in our patient, although we can not exclude abnormalities in other sex differentiation related genes.
There is a possibility that her menses were caused by peripheral aromatase activity or hypophysis-adrenal gland hormone in the absence of ovulation not under the control of ovarian hormone.
The patient's constitutional t(Y;15) is probably a balanced translocation derived from her

father which is also present in her brother without effect.

The authors thank Drs K Nishizawa and M Nakao for performing androgen receptor binding assays, Dr D C Page (MIT, Cambridge, USA) for providing probe pDP1007, and Dr Jean Weissenbach (Institute Pasteur, Paris, France) for probes 47z (DXYS5), 52d (DYF27A-C), 50f2 (DYS7A-E), 12f3 (DYS11), and 49f (DYS1). We also thank Ms Kadomi Monzaki, Yukiko Yamagami, Hiromi Shimakage, Yumi Gokan, and Miyuki Yasui for their technical assistance. This study was supported in part by a Grant-in-Aid for Scientific Research (No 03771069 and No 08671858) from the Ministry of Education, Science and Culture, Japan.

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