

Clinical, cytogenetic, and molecular analysis with 46,XX male sex reversal syndrome: case reports

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Abstract

Purpose To investigate the clinical characteristics of different categories of sex-reversed 46,XX individuals and their relationships with chromosomal karyotype and the SRY gene.

Methods Chromosome karyotyping for peripheral blood culture and multi-PCR and FISH were performed.

Results Endocrinological data showed that their endocrine hormone levels were similar to that observed for Klinefelter syndrome, with higher FSH and LH levels and lower T levels. Chromosome karyotyping for peripheral blood culture revealed 46, XX complement for 11 males. Molecular studies showed that there were locus deletions at SY84, SY86, SY127, SY134, SY254 and SY255 in AZF on chromosome Y in 9 cases, with the SRY gene present at the terminus of the X chromosome short arm. In one case, besides 6 locus deletions in AZF, there was also SRY gene deletion. In another case, there were locus deletions only at SY254 and SY255, with SY84, SY86, SY127 SY134 loci and SRY present.

Conclusions The majority (10/11) of 46,XX males were SRY positive, with the SRY gene translocated into the terminus of the X chromosome short arm. These patients were caused mainly by an X/Y chromosomal inter-change during paternal meiosis, leading to the differentiation of primary gonads into testes. Only a single patient (1/11)

was SRY-negative, in which there might be some unknown downstream genes involved in sex determination.

Keywords 46,XX male · Sex reversal · SRY gene

Introduction

Male sex determination has not yet been completely elucidated in humans. But there is agreement that the genes of the Y chromosome, in particular, the sex-determining region Y gene (SRY), plays a major role in encoding a testis determining factor (TDF) [1]. In XY embryos, SRY induces the gonadal primordium to develop into testis. 46,XX male syndrome is rare and its incidence is about 1:20000 in newborn males [2]. As far as the sexual phenotype is concerned, three clinical categories of sex-reversed 46,XX individuals have been identified: (1) Classic XX males, infertility with normal male internal and external genitalia; (2) XX males with ambiguous genitalia, usually detected at birth by external genital ambiguities such as hypospadias, micropenis, or hyperclitoridry; (3) XX true hermaphrodites, who carry internal or external genital ambiguities detected at birth [3–5].

At the molecular level, XX males can be classified as Y-DNA positive and Y-DNA negative, depending on the presence or absence of Y-specific sequences [6].

In this paper, the clinical characteristics, endocrinology, chromosomal karyotype and SRY gene were analyzed on eleven sex reversal males discovered during routine examinations for infertility, with the aim of investigating the clinical characteristics of different categories of sex-reversed 46,XX individuals and their relationships with chromosomal karyotype and the SRY gene.

Capsule Investigated the clinical characteristics of different categories of sex-reversed 46,XX individuals and their relationships with chromosomal karyotype and the SRY gene.

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Table 1 General characteristics and endocrine hormone levels

Subjects	Height cm	Volume of testes L/Rml	PRL ng/ml	FSH mIU/ml	LH mIU/ml	E2 pmol/l	T nmol/l
1	163	3/3	17.9	63.6	19.4	122	10.72
2	163	2/2	18.5	24.7	14.4	161	9.64
3	162	2/3					4.5
4	161	2/2	22.9	81.6	27.7	<73.4	4.78
5	158	2/4	9.67	13.1	3.61	126	8.49
6	162	2/2	10.08	54.7	19.4	101	6
7	162	3/3	9.88	37.1	16.5	105	11.1
8	161	3/2	7.28	43	33.9	81.1	7.5
9	160	2/2	10	72	34.6	<73.4	11.7
10	160	1/2	15.8	49.2	26.8	<73.4	6.28
11	161	2/2	49.6	87.7	31.4	112	18.1

Materials and methods

Subjects and clinical data

We retrospectively analyzed 11 married men with a history of several years of infertility, who were referred to us for genetic review. Their body heights were between 160 and 165 cm. The patients had complete outward signs of masculinity, with no symptom of undervirilization. Semen analyses showed azoospermia. Their testes were small in size and final clinical features were compatible with severe testicular atrophy. Their endocrine hormone levels were similar to those in patients with a chromosomal karyotype of 47, XXY. Ultrasonography failed to identify female gonads or female internal genitalia in all patients.

Karyotype analysis of G-banding in lymphocytes

G-banded chromosomes from cultured peripheral blood lymphocytes were analyzed with conventional methods. Briefly, whole blood (0.5 ml) was cultivated in RPMI-1640 containing PHA-M for 72 h at 37 °C, and then colchicine (1 µg/ml) was added to incubate for 30 min at 37 °C. Chromosomal preparations were carried out according to standard techniques. 100 metaphases were counted in all patients in order to exclude 46, XY mosaicism, with 3–5 karyotypes analyzed.

Polymerase chain reaction (PCR)

For analysis of Y chromosome specific regions, multiplex reactions were performed for the amplification of the six loci of the azoospermia factor (AZFa SY84, SY86; AZFb SY127, SY134 and AZFc SY254, SY255) regions on the Y chromosome and the SRY genes following the manufacturer's instructions. The multi-PCR kits were purchased from Toujing Life Technology Co., Ltd (Shanghai, China). The DNA samples of

normal men, normal women and double distilled water were used as positive, negative and blank controls, respectively.

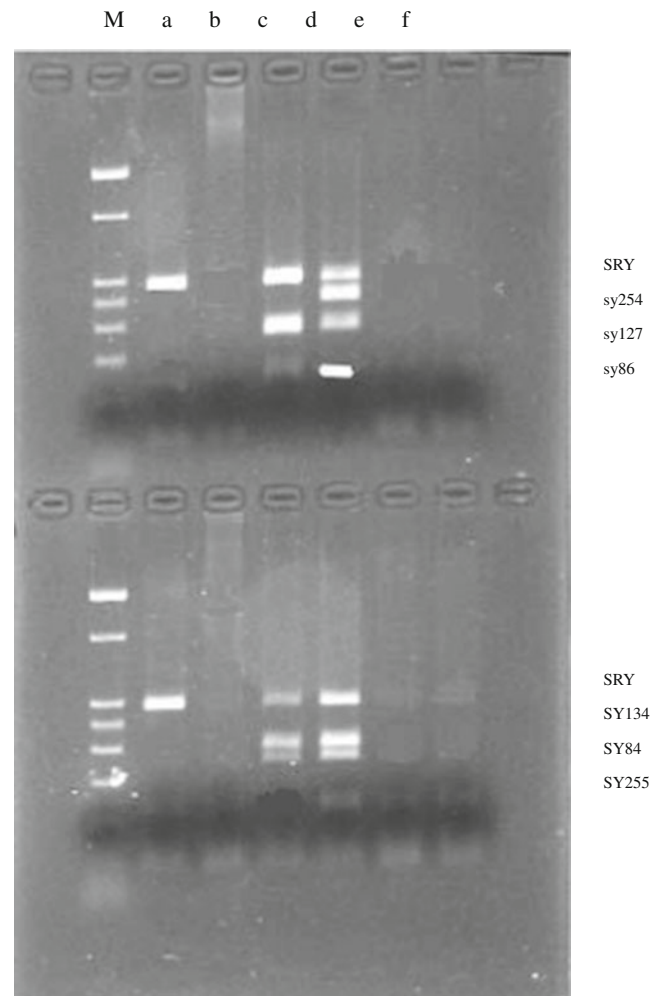


Fig. 1 PCR for SRY gene and the locus of AZF on chromosome Y. NOTE: M: marker a: 9th case b: 10th case c: 11th case d: normal male e: normal female f: blank control

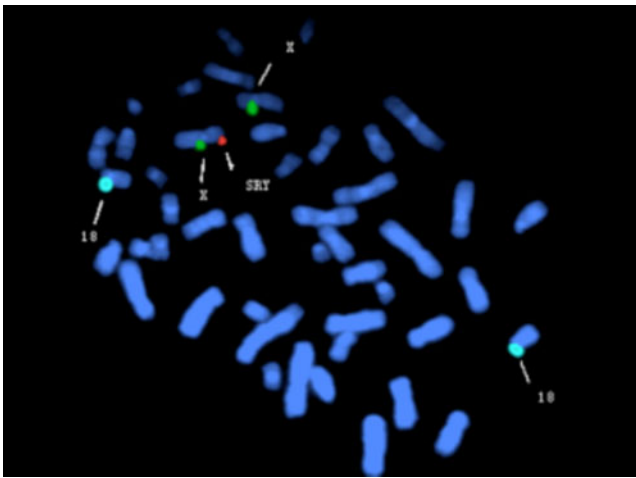


Fig. 2 The FISH on metaphase chromosomes of 9th case with LSI SRY(Yp11.3) in Orange hybridizing to the distal end of short arm of chromosome X

Fluorescence in situ hybridization (FISH)

FISH was performed in metaphase chromosome of peripheral blood lymphocytes with CEPX (DXZ1, Xp11.1-q11.1, Spectrum Green, Vysis), LSI SRY(Yp11.3, Spectrum Orange, Vysis) and CEP18 (D18Z1, 18p11.1-q11.1, Spectrum Aqua, Vysis) probes following the instructions provided by the manufacturer. 10–15 metaphases were analyzed in each sample using a Nikon fluorescence microscope for signals collection, with the chromosome mapping evaluated with CytoFish 5.6 software (United Scientific USA Inc).

Results

In 11 cases, the average body height was 161 cm and the average volume of testes 2.3 ml. Their endocrine hormone levels were similar to that observed in Klinefelter syndrome,

with higher level of FSH and LH and lower level of T. General characteristics and endocrine hormone levels are shown in Table 1.

Karyotype analysis of all patients showed apparent 46, XX chromosome complement and no evidence of mosaicism in peripheral blood cells.

Results of PCR and FISH

In 9 cases, there were locus deletions at SY84, SY86, SY127, SY134, SY254 and SY255 in AZF on chromosome Y, with the SRY gene present (Fig. 1a) and mapped to the distal end of the short arm of chromosome X (Fig. 2). In one case we found deletion of the SRY gene except 6 locus deletions in AZF (Fig. 1b), and another case had locus deletions only at SY254 and SY255, with SY84, SY86, SY127, SY134 loci and SRY gene all present (Fig. 1c). Details of PCR and FISH results are shown in Table 2.

Discussion

46,XX male sex reversal syndrome was first reported by Chapelle in 1964 [7]. Most patients (85 %) have a normal male phenotype at birth and are usually diagnosed after puberty during consultation because of hypogonadism, gynecomastia, and/or infertility [8]. Approximately 15 % of XX males are observed to have hypospadias, cryptorchidism, or severe genital ambiguity [9]. In present study, no abnormalities of urinal reproductive system were found in any patient, except for infertility with severe testicular atrophy.

Furthermore, their body heights were all under 165 cm (with an average of 161 cm), which is likely due to the lack of an appropriate T-dependent pubertal growth spurt [10] and Y-specific growth gene(s) mapped in the pericentromeric region of the Y chromosome [11].

Table 2 Examination of STS locus and SRY gene on Y chromosome

	SY84	SY86	SY134	SY127	SY254	SY255	SRY	
							PCR	FISH
1	-	-	-	-	-	-	+	Xp
2	-	-	-	-	-	-	+	Xp
3	-	-	-	-	-	-	+	Xp
4	-	-	-	-	-	-	+	Xp
5	-	-	-	-	-	-	+	Xp
6	-	-	-	-	-	-	+	Xp
7	-	-	-	-	-	-	+	Xp
8	-	-	-	-	-	-	+	Xp
9	-	-	-	-	-	-	+	Xp
10	-	-	-	-	-	-	-	-
11	+	+	+	+	-	-	+	Xp

SRY is considered a genetic switch directing the identity of the bipotential gonadal primordium to testes [12]. Molecular genetics analyses have demonstrated that approximately 90 % of these patients carry a variable amount of Y material due to a Y-to-X interchange caused by an illegitimate recombination during paternal meiosis [13–15]. There was a report of an XX male caused by a translocation of an SRY gene fragment from the Y chromosome to an autosome [16].

In contrast, there are about 10 % of XX males whose testicular differentiation occurs in absence of detectable Y sequences [8, 13, 17–21]. The majority of these patients have a variable degree of ambiguous external genitalia although a complete male phenotype could also be observed [8, 15]. That would be considered as evidence supporting the theory that the SRY gene does not function alone in the determination of the male phenotype, but disruption of an unidentified autosomal or X-linked sex-determining gene may interact in the sex-determining cascade [13, 18].

Furthermore, the DAX1 and SOX9 genes might function downstream of the SRY gene in the sex-determination pathway. Any mutation leading to increased expression of either gene could lead to female-to-male sex reversal in 46,XX SRY-negative individuals [22, 23]. Huang et al. reported a 46,XX sex reversal individual with duplication of chromosome 17q including SOX9 [24], which suggested that overexpression of male determining genes could trigger testis determination in the absence of SRY. The observation of male development on SOX9 transgenic XX mice further confirmed this hypothesis and raised the possibility that some SRY-negative XX males might have a microduplication of chromosome 17q involving the SOX9 gene [25]. Other study on an individual with SRY-negative 46,XX true hermaphroditism who had a duplication of the long arm of chromosome 22 from q13.1 to qtelomere also suggested that the existence of certain genes on 22q play a fundamental role in testis determination [26]. However, Tosaporn et al. [27] investigated 13 subjects with SRY-negative 46,XX sex reversal for microduplication of chromosome 22q in the region of SOX10 gene, but failed to find any evidence for this. To explain the induction of testicular tissue in SRY-negative patients, another hypothesis suggesting the presence of hidden mosaicism containing Y-derivative material has been proposed [2]. But there has been few reports to prove it on XX-reversed patients as of yet. In this study, no mosaicism containing Y-derived material was detected in all 11 cases with 46,XX male sex reversal, at least in the lymphocytes.

In present study, the presence of the SRY gene were confirmed in 10 out of 11 cases both by PCR and FISH, with the SRY gene located at the short arm of chromosome X, but translocation of SRY to autosome was not found. In our opinion, the illegitimate X/Y chromosomal interchange during paternal meiosis would result in the differentiation of primary gonads into testes in 46,XX SRY-positive male. 46,XX SRY-negative individuals with complete masculinization

are rare [8, 13, 17–19]. Here, we reported one case of 46,XX SRY-negative male with full masculinization but infertility.

In our study, all patients showed azoospermia, which might be related to absence of the Y chromosome long arm which contains three AZF regions [28]. Furthermore, many genes related to spermatogenesis are known to be harbored in these regions, such as USPY9 and DBY in AZFa, RBMY1 in AZFb, and DAZ in AZFc.

Except for SY254 and SY255, some STS loci in AZF including SY84 and SY86 in AZFa, SY127 and SY134 in AZFb, were all present in one out of 11 cases who had normal limits of androgen levels but azoospermia. The possible explanation might be that besides the translocation of SRY gene fragment on the short arm of chromosome X, the translocations of other related genes on the long arm of chromosome Y would also interfere with the function of genes related to spermatogenesis on the area.

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