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Clinical Report

True Hermaphroditism With Ambiguous Genitalia Due to a Complicated Mosaic Karyotype: Clinical Features, Cytogenetic Findings, and Literature Review

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Abnormal recombination between the X and Y chromosomes during meiosis, occurring outside the pseudoautosomal region, can result in translocation of the *SRY* gene from the Y to the X chromosome, and consequently in abnormal sexual differentiation, such as the development of 46,XX males or true hermaphroditism. In this report we present clinical, cytogenetic, and molecular-cytogenetic data of a patient with ambiguous genitalia and true hermaphroditism, who had a unique mosaic karyotype, comprising three different cell lines: 46,XX^{SRY+}, 45,X^{SRY+}, and 45,X. The mosaic karyotype of our patient probably represents two different events: abnormal recombination between the X and Y chromosomes during paternal meiosis, and postzygotic loss of one of the X chromosomes. Replication studies demonstrated that in 80% of the XX cells, the *SRY* sequence was located on the active X chromosome. This finding suggests nonrandom X inactivation and, together with the presence of the *SRY* gene, explains the male phenotype of our patient. On the other hand, the presence of the 45,X cell line may have contributed to genital ambiguity. We conclude that fluorescence in situ hybridization (FISH) analysis with *SRY* probes is highly recommended and allows accurate diagnosis and optimal management in cases of 46,XX hermaphroditism and ambiguous genitalia.
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KEY WORDS: true hermaphroditism; ambiguous genitalia; XX male; mosaicism; FISH analysis

INTRODUCTION

Sex determination and differentiation are sequential processes regulated by an unknown number of gene loci located on sex and autosomal chromosomes that occur in the testis-determining pathway. However, there is wide evidence that the Y-located *SRY* gene triggers testes formation from the undifferentiated gonad [Canto et al., 2000]. Organizing factors, gonadal steroids, peptide hormones, and tissue receptors are also involved in sex determination; interruption at any level will cause a sex differentiation disorder.

The human Y chromosome can be divided into recombining and nonrecombining regions: the pseudoautosomal regions contain genes with homologues on the X chromosome, and the nonrecombining regions carry genes that are mostly male specific, and are considered to have a role exclusively in testis determination and male germ cell development [Cooke, 1999].

Recombination between the X and Y chromosomes occurs during meiosis and is normally restricted to the pseudoautosomal regions located at both ends of the sex chromosomes, where pairing of X and Y chromosomes also initiates. An obligate crossover synapsis in the pseudoautosomal region (PAR1) during male meiosis seems to be necessary for male fertility. Recombination occurring outside the pseudoautosomal region can result in a translocation of the *SRY* gene from the Y to the X chromosome, and consequently in abnormal sexual differentiation, such as the development of 46,XX males [Schiebel et al., 1997].

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46,XX maleness (testicular development and masculinization of an apparently XX subject) occurs in about 1/20,000 male neonates. Most of these subjects have normal male external genitalia, but 10% of them have hypospadias and all are infertile [Fechner et al., 1993]. The majority of subjects with 46,XX maleness have *SRY* containing Y chromosome sequences translocated to the distal region of the paternal X chromosome (Xp22.3 to Xpter). Some XX males also have been reported lacking any Y sequences. These individuals usually have sexual ambiguities or hermaphroditism or both. These patients may have Y sequences confined to testicular tissue, therefore escaping detection by the analysis of leukocyte DNA, or alternatively, they may have mutations in genes controlling testicular development downstream to *SRY* [Fechner et al., 1993; Margarit et al., 1998]. The different phenotypes of XX males and true hermaphrodites who are carriers of the same translocation may also be explained by a different pattern of inactivation of the Y-bearing X chromosomes [Margarit et al., 2000].

In this paper we present clinical, cytogenetic, and molecular-cytogenetic data of an unusual patient with an apparent 46,XX/45,X karyotype and male sexual development.

CLINICAL REPORT

The patient was the product of a clomiphene-induced pregnancy. Prenatal ultrasonography revealed a female phenotype. Delivery was induced at 36 weeks because of intrauterine growth retardation and oligohydramnios. Birth weight was 1,400 g. After birth, ambiguous genitalia were noted: small phallus measuring 2×1 cm, grade IV hypospadias with chordee, and a bifid scrotum with gonads palpable in both labioscrotal folds. No Mullerian structures were visualized by ultrasonography or computed tomography of the pelvis. Laboratory studies revealed normal testosterone and gonadotropins with good response to hCG administration (Table I).

Because of the appearance of the external genitalia, and as both gonads were palpable in the scrotum and there was evidence of good testicular function, male sex of rearing was assigned.

The patient underwent surgery for correction of hypospadias at age 22 months. During surgery, Mullerian structures were noted. A biopsy of both gonads was performed, identifying one gonad as a testis and the other as an ovary. The penis was of normal size (3.0×1.0 cm). The patient's psychomotor development is normal for his age.

MATERIALS AND METHODS

Cytogenetic Analysis

Lymphocyte cultures were obtained from the patient, and at least 15 metaphase spreads were analyzed by standard trypsin-Giemsa (GTG) staining (G banding).

Fluorescent In Situ Hybridization (FISH)

FISH experiments were performed on metaphase spreads derived from the patient's lymphocytes, according to the Vysis (Naperville, IL) protocols for direct probes. Slides were counterstained with 80 μl of 4′-6 diamino-2-phenylinodole (DAPI) (10 g/ml).

Hybridizations were performed by the use of LSI *SRY* (Yp11.3) Spectrum Orange, CEP X Spectrum Green, CEP Y (α-Satellite) Spectrum Orange, and CEP Y Sat III: Spectrum Orange (Vysis, Naperville, IL).

Microscopy

Bright light and fluorescent microscopy was performed with a Zeiss Axiscope Microscope (Zeiss, Jena, Germany). A triple filter set (Vysis, Naperville, IL) for simultaneous detection of DAPI, Spectrum Orange, and Spectrum Green was used to visualize red and green signals. Several images were processed using Cytovision-automated system (Applied Imaging, Santa Barbara, CA). Each slide was scored by at least two observers.

Replication Timing Analysis

In female somatic cells, the inactive X chromosome replicates in the late S phase. Late replication timing is frequently used as a criterion for identifying the inactive X and for determining the inactive status of autosomal regions translocated onto the X chromosome [Heard et al., 1997]. Using FISH to interphase nuclei, we examined replication timing of one allele relative to its counterpart. In the FISH assay an unreplicated DNA sequence is identified by a single dot-like hybridization signal, whereas a replicated region gives rise to a duplicated, bipartite signal. Following FISH with CEP X Spectrum Green and *SRY* Spectrum Orange (Yp11.3), we analyzed interphase cells that showed two different X-centromeric hybridization signals. Cells were classified

TABLE I. Laboratory Data

	Units	48 hours	9 days	12 days[a]	10 months
17-β E2	pg/ml		33.3		
Testosterone	ng/ml	2.2	1.7	4.9	<0.5
LH	MIU/ml	<1	16.9	7.7	<0.6
FSH	MIU/ml	<1	4.9	3.8	<0.3
17-OH-Progesterone	ng/dl	0.3			
DHEA-S		1.5			
Androstendione	ng/dl	2.8			
FT4	pmol/L	34.9			
TT3	nmol/L	2.3			
TSH	mU/L	11.5			

[a]After stimulation with HCG.

into three categories: 1) cells with two signals (singlet, singlet), representing cells where both sequences are unreplicated; 2) cells with one singlet and one doublet, revealing S-phase cells where only one of the sequences has replicated; 3) cells with two doublets, representing cells in which both sequences have replicated.

To identify the active X chromosome, 199 informative interphase cells, with one singlet and one doublet signals, were scored.

RESULTS

Cytogenetic Analysis

G-banded chromosome spreads derived from peripheral lymphocytes revealed a 46,XX/45,X (90%/10%) mosaic karyotype.

FISH Analysis

FISH analysis on interphase blood cells, using an X centromere probe and a Y heterochromatic region probe (CEP Y Sat III; Spectrum Orange), demonstrated X chromosome mosaicism of 46,XX/45,X (85%/15%). No Y centromeric signal was observed. Additional FISH analysis, on metaphase spreads, using the *SRY* probe labeled with Spectrum Red and the X centromere probe labeled with Spectrum Green, demonstrated *SRY* signals located on the short arm of chromosome X.

Among the metaphase cells with 46,XX karyotype, 90% of the cells demonstrated an *SRY* signal (the 10% that did not exhibit signals of *SRY* are considered to be in the range of the method's fault). Among the metaphase cells with 45,X karyotype, 86.2% demonstrated an *SRY* signal and in 13.8% the *SRY* gene was not detected.

Replication Timing Analysis

In order to identify the active and inactive X chromosomes, replication timing of the X α-Satellite centromeric region in the patient's lymphocytes was studied. Only cells with two X-centromeric signals and one *SRY* signal were scored. We analyzed cells having both a nonreplicated locus (singlet signal (S)) and a replicated locus (doublet signal (D)). Such cells, showing a singlet/doublet (SD) hybridization pattern, are informative, and one can easily detect the active (early replicating) and the inactive (late replicating) X chromosomes.

In 80% of the XX cells, the *SRY* sequence was located on the active X chromosome.

DISCUSSION

In this study, we present a male patient with a unique mosaic karyotype, comprising three different cell lines, namely, $46,XX^{sry+}$, $45,X^{sry+}$, and 45,X. This mosaicism is probably the result of two events. The first is an abnormal exchange between the X and Y chromosomes during the first male meiotic division, resulting in translocation of the *SRY* gene to the short arm of the X chromosome. The second is loss of either one of the X chromosomes during early embryonic development. Phenotypically, the patient has a normal size penis and true hermaphroditism (one gonad is identified as a testis and the other as an ovary). While 46,XX/45,X is one of the most common forms of mosaicism, the distinguishing factor in this case is the presence of the *SRY* gene.

Translocation of Y sequences to the X chromosome usually results in 46,XX maleness, with a phenotype similar to that of patients with Klinefelter syndrome: normal male external genitalia, small testes, gynecomastia, sterility owing to Sertoli cell dysfunction, and partial Leydig cell dysfunction [Fechner et al., 1993; Margarit et al., 1998]. However, unlike patients with Klinefelter syndrome, XX males are shorter than normal XY males, probably due to absence of Yq-specific growth genes [Ferguson-Smith et al., 1990; Ogata and Matsuo, 1992]. Since the distal long arm of the Y chromosome (Yq11) contains a number of genes regulating spermatogenesis, including *DFFRY* (Drosophila fat-facets related Y), *RBM* (RNA-binding motif), and *DAZ* (deleted in azoospermia) (reviewed by McElreavey and Krausz [1999]), azoospermia would be expected in Xp/Yp translocation. Since the postulated gonadoblastoma gene (*GBY*) is located near the Y centromere, these patients should have a much lower risk for gonadoblastoma compared to other patients with Y chromosome aneuploidy, and gonadectomy is probably not indicated in them [Hsu, 1994; Lau, 1999].

Some subjects with 46,XX maleness present with genital ambiguity or even true hermaphroditism [Berkovitz et al., 1992]. It was suggested that the clinical phenotype may depend on the length of the translocated Y material, with longer Yp fragments resulting in a more complete masculinization of the external genitalia [Abbas et al., 1990; Ferguson-Smith et al., 1990; Kusz et al., 1999]. Another theory is that extensive inactivation of the *SRY*-carrying X chromosome may be the major mechanism causing sexual ambiguity, including the presence of ovarian tissue, in XX (*SRY*+) subjects [Kusz et al., 1999; Margarit et al., 2000]. Kusz et al. [1999] reported that in their patients with XX true hermaphroditism, the *SRY* gene was translocated onto an inactive X chromosome in over 90% of their blood lymphocytes, whereas in an XX male with no ambiguous features, the *SRY* gene was translocated onto the active X chromosome in over 90% of cells. In our patient, the *SRY* sequence was located on the active X chromosome in 80% of the XX lymphocytes, suggesting nonrandom X inactivation, yet masculinization was incomplete. This can be explained either by a different pattern of X inactivation in the gonads (compared to lymphocytes) or by the presence of the 45,X cell line.

In a review of 228 cases with Y chromosome aneuploidy with mosaicism for a 45,X cell line [Hsu, 1994], it was found that the presence of a 45,X cell line tended to be associated with a female phenotype with streak gonads and Turner's features or with genital ambiguity. Moreover, the percentage of 45,X cell lines was clearly higher among the females and in the patients with ambiguous genitalia than in the phenotypic males [Hsu, 1994]. Thus, the 45,X cell line (15%) found in our patient is probably the cause of his incomplete masculinization.

Two patients with a karyotype similar to that of our patient were previously reported. Both had ambiguous external genitalia. Osipova et al. [1998] described a patient with a 45,X/46,XX (67%/33%) karyotype who

was *SRY* positive. Since the presence of *SRY* was identified by polymerase chain reaction (PCR), and FISH analysis was not performed, it is not known whether all the cells of the patient contained the *SRY* sequence on their X chromosomes. The patient is described as having classic Turner syndrome features, a normal-length phallus, scrotal hypospadias, and bilateral testes [Osipova et al., 1998]. Kusz et al. [1999] described a patient with a 46,XX^{SRY+}/45,X (8%) karyotype. The clinical phenotype was true hermaphroditism with ambiguous external genitalia [Kusz et al., 1999].

Regarding the mechanism of origin of the 45,X karyotype, in the majority of patients, the missing X is lost through nondisjunction during paternal meiosis. However, in the present case, the 45,X cell line probably resulted from loss of either one of the X chromosomes during early embryonic development.

In summary, the patient described in this study is a true hermaphrodite with ambiguous genitalia due to the complicated mosaic karyotype. Translocation of the *SRY* region onto the X chromosome followed by selective inactivation of the maternal X chromosome contributed to the male phenotype. At the same time, the presence of a 45,X cell line probably prevented complete masculinization and resulted in genital ambiguity and the presence of ovarian tissue.

Since it is very important to identify the presence of Y sequences on the X chromosome in cases of 46,XX hermaphroditism and ambiguous genitalia, application of FISH analysis with *SRY* probes is highly recommended. The FISH technique is very useful for detecting and locating Y sequences in cytogenetically XX phenotypic males, allowing accurate diagnosis and appropriate management of the patient. Testing new Y chromosome markers in XX males will make it possible to narrow the breakpoints further in each case and to establish correlations with the clinical features, identifying the Y regions implicated in the definition of the phenotype.

REFERENCES

Abbas NE, Toublanc JE, Boucekkine C, Toublanc M, Affara NA, Job JC, Fellous M. 1990. A possible common origin of "Y-negative" human XX males and XX true hermaphrodites. Hum Genet 84:356–360.

Berkovitz GD, Fechner PY, Marcantonio SM, Bland G, Stetten G, Goodfellow PN, Smith KD, Migeon CJ. 1992. The role of the sex-determining region of the Y chromosome (*SRY*) in the etiology of 46,XX true hermaphroditism. Hum Genet 88:411–416.

Canto P, de la Chesnaye E, Lopez M, Cervantes A, Chavez B, Vilchis F, Reyes E, Ulloa-Aguirre A, Kofman-Alfaro S, Mendez JP. 2000. A mutation in the 5′ non-high mobility group box region of the *SRY* gene in patients with Turner syndrome and Y mosaicism. J Clin Endocrinol Metab 85: 1908–1911.

Cooke HJ. 1999. Y chromosome and male infertility. Rev Reprod 4:5–10.

Fechner PY, Marcantonio SM, Jaswaney V, Stetten G, Goodfellow PN, Migeon CJ, Smith KD, Berkovitz GD, Amrhein JA, Bard PA, et al. 1993. The role of the sex-determining region Y gene in the etiology of 46,XX maleness. J Clin Endocrinol Metab 76:690–695.

Ferguson-Smith MA, Cooke A, Affara NA, Boyd E, Tolmie JL. 1990. Genotype-phenotype correlations in XX males and their bearing on current theories of sex determination. Hum Genet 84:198–202.

Heard E, Clerc P, Avner P. 1997. X-Chromosome inactivation in mammals. Annu Rev Genet 31:571–610.

Hsu LY. 1994. Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. Am J Med Genet 53:108–140.

Kusz K, Kotecki M, Wojda A, Szarras-Czapnik M, Latos-Bielenska A, Warenik-Szymankiewicz A, Ruszczynska-Wolska A, Jaruzelska J. 1999. Incomplete masculinisation of XX subjects carrying the *SRY* gene on an inactive X chromosome. J Med Genet 36:452–456.

Lau YC. 1999. Gonadoblastoma, testicular and prostate cancers, and the TSPY gene. Am J Hum Genet 64:921–927.

Margarit E, Soler A, Carrio A, Oliva R, Costa D, Vendrell T, Rosell J, Ballesta F. 1998. Molecular, cytogenetic, and clinical characterisation of six XX males including one prenatal diagnosis. J Med Genet 35:727–730.

Margarit E, Coll MD, Oliva R, Gomez D, Soler A, Ballesta F. 2000. *SRY* gene transferred to the long arm of the X chromosome in a Y-positive XX true hermaphrodite. Am J Med Genet 90:25–28.

McElreavey K, Krausz C. 1999. Sex Chromosome Genetics '99. Male infertility and the Y chromosome. Am J Hum Genet 64:928–933.

Ogata T, Matsuo N. 1992. Comparison of adult height between patients with XX and XY gonadal dysgenesis: support for a Y specific growth gene(s). J Med Genet 29:539–541.

Osipova GR, Karmanov ME, Kozlova SI, Evgrafov OV. 1998. PCR detection of Y-specific sequences in patients with Ullrich-Turner syndrome: clinical implications and limitations. Am J Med Genet 76:283–287.

Schiebel K, Winkelmann M, Mertz A, Xu X, Page DC, Weil D, Petit C, Rappold GA. 1997. Abnormal XY interchange between a novel isolated protein kinase gene, PRKY, and its homologue, PRKX, accounts for one third of all (Y+)XX males and (Y−)XY females. Hum Mol Genet 6:1985–1989.