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CASE REPORT

A case report of an XX male with complete masculinization but absence of the SRY gene ☆

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Abstract A 34-year old man with complete masculinization and a history of several years of infertility was referred to us for genetic reviewing. His semen analysis showed azoospermia. Conventional chromosomal analysis indicates a 46,XX karyotype, molecular analyses excluded the presence of SRY (the sex-determining region of the Y chromosome) gene. This case is one of the rare cases reported in the literature in whom testicular differentiation and complete virilization were found in a 46,XX chromosomal constitution, with the absence of SRY gene. This finding suggests that other genes downstream from SRY play an important role in sex determination. Through reporting this rare case and reviewing previous literatures, the aim of this report is to highlight the value of genetically screening all males with azoospermia who present for evaluation of infertility, since the phenotype does not always correlate with the genotype.

1. Introduction

The process of sex determination in humans has not yet been completely elucidated (1). However, it is clear that this process involves a gene regulatory network in which a gene, located on Y-chromosome Yp11.32, named SRY (sex-determining region of the Y), plays a crucial role (2). SRY induces the undifferentiated embryonic gonad to develop as a testis (3). Although its specific function and downstream molecular targets remain largely unknown. Based on karyotype analysis and detection of SRY gene, 46,XX male patients can be divided into the SRY positive and the SRY negative groups. While the clinical symptoms of patients often show some degree of heterogeneity usually, the development of genitalia is normal and masculinity signs are obvious in SRY gene-positive patients, in contrary, most SRY gene-negative patients could be easily discriminated due to abnormality of genitalia shortly after birth; some patients even show genital ambiguity (4,5). In addition, masculinity signs in most cases are not clear in SRY gene-negative patients; especially in adult patients, breast development and the female secondary sex characteristics can

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be found. The presence of male cases negative for the SRY gene with evident malformation in their extra genitalia suggests that the SRY gene is a key in sex determination and development, yet there might be other important genes involved (6) specially with an increasing number of reports suggest that the male phenotype can develop even in the absence of SRY gene (7). In this study, we report a rare case of XX male with SRY-negative, having full masculinization but with infertility.

2. Material and methods

2.1. Subject and clinical features

A thirty-four-year old married man with a history of several years of infertility was referred to us for genetic reviewing. Semen analyses showed azoospermia, hormone profile of the patient showed an elevation of the follicle-stimulating hormone (FSH) 29 mIU/ml (normal range 1.5–15.0 mIU/ml), while the serum concentration of the testosterone and the Anti-Mullerian (AMH) hormones were low, 3 nmol/l (normal range 8.3–38.2 mIU/ml) and 0.19 ng/ml (normal range 2.0–5 ng/ml), respectively. The luteinizing hormone (LH) was normal 6 mIU/ml (normal range is 1.4–7.7 mIU/ml). The patient had fully mature normal male genitalia with no symptom of undervirilization. The testes were descended in the scrotum, but small in size and his final clinical features were compatible with severe testicular atrophy.

2.2. Cytogenetic analysis

Peripheral blood lymphocyte cultures were set up using special medium for human lymphocyte culture. Dividing cells were arrested at metaphase stage and fixed. Chromosomes were studied by GTG method. A total of 50 metaphases were analyzed to look at any numerical or structural chromosomal aberrations.

2.3. Fluorescent in situ hybridization (FISH) of the SRY gene

To check for the possible presence of SRY, FISH analyses on metaphase cells from peripheral blood were performed, using (LSI SRY SO/CEP X SG dual color DNA probe Vysis®) that hybridizes to band Yp11.3 of the human Y chromosome (LSI SRY spectrum orange), and to the centromere, band region Xp11.1-q11.1 locus DXZ1 (CEP X spectrum green) of the human X chromosome. Fluorescence microscope (Nikon Eclipse E800 Japan) was used to observe and analyze the results.

2.4. Molecular analysis study of the SRY and Y-chromosome-specific markers

The genomic DNA was extracted from the peripheral blood lymphocytes of the patient, a normal human fertile male (positive control) and a normal human female (negative control). To exclude the presence of any Y material and the absence of the SRY gene we performed a PCR multiplex method amplification, using 11 sequence-tagged sites (STS) which are Y-chromosome-specific markers for spermatogenic genes in the azoospermic locus (AZF), a primer to amplify the SRY gene, also a special primer to amplify a unique fragment in both Y and X chromosomes of the gene ZFY/ZFX, respectively. The PCR products were electrophoresed on 2.5% agarose gel containing ethidium bromide (EB) and observed in ultraviolet projector and photographed.

3. Results

Analysis of 50 metaphases showed a constitution of 46,XX genotype with no evidence of mosaicism, structural or numerical chromosomal abnormalities.

FISH analysis detected two green signals of the X chromosome only and no signal for SRY could be seen in all the 100 nuclei and metaphases studied.

Molecular analysis of Y-chromosome-specific markers, SRY and ZFY revealed their absence in the patient's DNA, however, the presence of the ZFX appeared clearly. All amplification products were found in the male positive control, amplification of the female genomic DNA failed to reveal any of the markers, or the SRY gene, however, it revealed the amplification of the ZFX gene only (Fig. 1). These results confirm that the patient is an XX male, with absence of the SRY gene, and without any material of the Y chromosome.

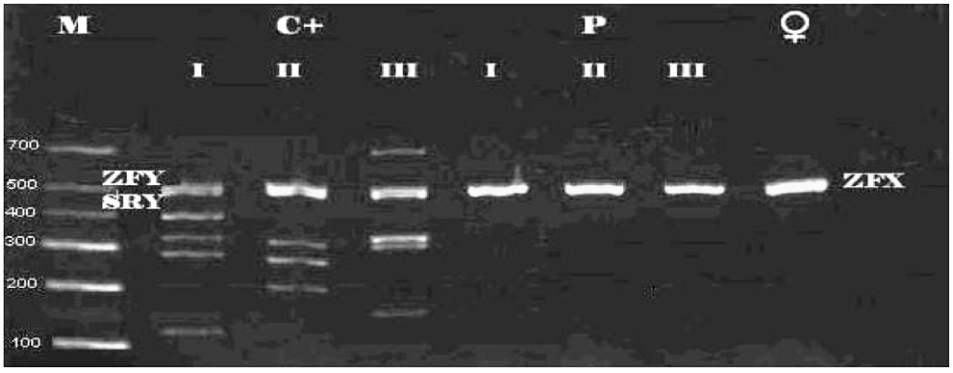

Figure 1 Gel electrophoresis of the PCR products of the STS markers, SRY and ZFY/ZFX genes. Lane M 100 bp ladder. C+ Lanes I–II–III normal fertile male. P Lanes I–II–III the patient. ♀ normal female. The 472 bp amplification of the SRY gene could be seen in the normal fertile male (C+ Lanes I–II–III) along with all the STS markers, whereas neither the patient nor the normal female had such fragments. Only the amplification of the ZFX gene could be seen at the patient and normal female lanes.

4. Discussion

XX male syndrome is a rare syndrome usually occurs as a sporadic event with a frequency of approximately 1/20,000–1/25,000 individuals. The vast majority, about 90%, has SRY detectable in their cells, the remaining 10% are SRY negative (8), although some research suggest a higher figure that reaches up to 20% of the cases (9). The cause of XX male negative for SRY gene is not known. It is well recognized that the presence of the SRY gene determines the sex in mammals by way of directing the sex determination pathway towards male development (10) but the existence of SRY-negative males ruled out the prevailing notion that the mere presence of SRY determines maleness (7), and the development of the testis and normal male genitals in a significant number of SRY-negative 46,XX males gives clue to the existence of other autosomal or X-linked genes in the sex-determining pathway (11). The etiology of development of male phenotype in most of the SRY-negative 46,XX males remains unexplained, researchers (12,13) suggested the presence of other mutations (autosomal or X-linked) which could be responsible for testicular determination in the absence of Y sequences. SOX9 and DAX1 genes have recently been proposed to function downstream to SRY gene in male sex determination pathway (14). The reporting of a Mexican family in which two siblings without genital ambiguities were SRY negative (4), suggested that an inherited loss of function mutation in a gene participating in the sex-determining cascade could induce normal male sexual differentiation in the absence of SRY gene. Another possibility for the etiology of maleness in these cases is a downstream gene on the X chromosome in which expression is influenced by X inactivation (15). Lastly, it has been postulated that the sex reversal in these patients is due to a defect on a yet unidentified autosomal or X-linked sex-determining gene (16). If the gene is autosomal, the degree of the male phenotype will be dependent on the extent of the loss or gain of function in the mutant gene. The phenotype in the heterozygotic mutants for X-linked gene will be determined by the ratio of the active and inactive copies of the gene (8). But until date, no clear explanations have been reached. Comprehensive genetic analysis of similar cases may help to detect new gene(s) involved in the sex-determining pathway.

This case is one of the rare cases we examined amongst the 262 patients, who came to our laboratory for genetic reviewing, this SRY-negative XX male have fully mature normal male genitalia with infertility as the main complaint. Cytogenetic and molecular cytogenetic analysis showed only 46,XX cell populations without any numerical or structural chromosomal aberrations. Molecular study was negative for SRY gene and other Y-chromosome sequences. We conclude that the presence of such cases emphasise the importance of genetic evaluation of all infertile couples seeking reproductive assistance since not always the phenotype does correlate with the genotype.

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