An Infertility SRY-Negative 46, XX Male Detected by Quantitative Fluorescent Polymerase Chain Reaction

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Abstract

Taking a frequency of 1 in 20,000–25,000 males, De la Chappelle syndrome (46, XX male) is a rare syndrome which exists in different clinical categories with ambiguous genitalia or partially to fully mature male genitalia, in combination with complete or incomplete masculinization. A case of SRY-negative XX male is reported with complete masculinization but with infertility. The patient was mature enough to have full genitalia with descended but small testes and no signs of undervirilization. There were 14 markers in the quantitative fluorescent polymerase chain reaction (QF-PCR): amelogenin gene, presented on X and Y chromosomes allows for the determination of the Y/X ratio (AMEL marker), TAF9B gene presented on chromosomes X and 3 permits the determination of X/chromosome 3 ratio (TAF9B marker or T3 marker), ten non-polymorphic Y-specific markers (SRY, sY84 and sY86 in AZFa region, sY127 and sY134 in AZFb region, sY254, sY255, sY1191, sY1192 and sY1291 in AZFc region), and co-amplification of DAZ/DAZL and CDY2/CDY1 fragments that permit determination of the DAZ, CDY1 and CDY2 gene copy number. The study resulted that there were only two peaks of markers: AMELX/Y marker produced a peak area ratio of 1.0 and T3 with the ratio of 2.2, indicating the presence of two X chromosomes in this case. The absence of the Y chromosome was confirmed when repeating peripheral blood culture for chromosome: there are 46 chromosomes with XX constitution. The first reported case in Mekong Delta, Vietnam is reported of a SRY-negative 46, XX male with normal male phenotype and infertility.

Keywords: XX male; Infertility; SRY gene; QF-PCR

Introduction

In 1964, Chappelle et al. described the first case of sex-reversed 46, XX individuals [1], classified in three groups: the first one is males who are phenotypically normal XX (also known as 46, XX testicular disorder of sex development), who are usually present with primary infertility, hypospadias and cryptorchidism. They are unambiguous males having normally differentiated with internal and external genitalia. The second group consists of males with genital ambiguities and the third group describes true hermaphrodites. They usually demonstrate ambiguous external and internal genitalia and have ovary and/or testes and/or ovotestes as the gonads [2,3]. There are ninety percent of these patients having Y chromosomal material including the SRY (Sex determining region of Y chromosome) gene [4]. The rest of XX males are SRY-negative with different degrees of masculinization [5]. There has not been a relationship between XX male prevalence and paternal age [6]. Azoospermia combined with one or more of abnormal external genitalia, gynecomastia, short stature and pelvic cyst are the clinical features of male sex reversal syndrome patients. There have been some reports describing the patients having male sex reversal syndrome with normal male phenotype without SRY gene [7-11]. This 46, XX male syndrome is regarded as a rare cause of male infertility-a 46, XX male but with no SRY gene on the X chromosome.

Among 46, XX males, approximately 10% of whom have no SRY gene [12]. These males are infertile [8,13] and most have ambiguous external genitalia [14-16]. In some cases, a normal male phenotype is seen [17]. Until now, the mechanism resulting in SRY-negative 46, XX males are unknown. Mutations may exist in an unknown X-linked or autosomal gene involved in testis differentiation, or a hidden Y chromosome mosaicism limited to the gonad has been suggested [18-21]. A few cases are to duplicate SOX9 which is sufficient to initiate testis differentiation in absence of SRY [22]. The same occurs with partial duplication of chromosome 22q, or with over expression of SOX10 at 22q13, mutations in RPSPO1 gene, translocation between chromosome 12 and 17, and SOX3 gain-of-function mutation [21,23,24]. Tests show diffuse tubular hyalinization and Leydig cell hyperplasia [25].

Case Report

A man in this study is 33-year-old, with the height of 169 cm and the weight of 58 kg. He was married and had fully mature normal male genitalia with no symptom of undervirilisation with normal density and distribution of axillary and pubic. He was referred to our infertility outpatient unit with a 2-year history of male factor infertility. His soft and small testis were palpated. Besides, semen analysis was carried out according to the World Health Organization manual (5th Edition). Azoospermia appeared in semen analysis. Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) were about 16.8 mIU/ml and 46.16 mIU/ml, respectively (normal ranges are 20 to 10 mIU/ml and 1.5 to 20 mIU/ml, respectively). The serum testosterone concentrated 3.23 ng/ml (in case normal ranges are 1.5 to 6.6 ng/ml). The ultrasound testicular volumes were calculated using the formula length × width × height × 0.71. The Prader orchid meter was used to measure the volume of testes by ultrasonography. Scrotal ultrasound scanning showed, both testes were small with right testes 15 mm × 6 mm × 8 mm, and left

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testes (17 mm × 6 mm × 8 mm), and both are in the scrotum. The mean volume by orchidometry was 2 mL for the right testis and 2 mL for the left smaller than Vietnamese adult male was 17.2 ± 5.4 mL [26].

Using 14 markers: AMEL, TAF9B (T3), SRY, CDY, DAZ, sY84, sY86, sY127, sY134, sY254, sY255, sY1191, sY1192, sY1291 in quantitative fluorescence polymerase chain reaction (QF-PCR) analysis resulted of no Y chromosome in his karyotype. The AMEL is a non-polymorphic (no-STR) marker present on both X and Y chromosomes. The relative amount of AMELX and AMELY products for individuals with 46, XY would expected to produce two peaks with a ration of 1:1. For AMEL marker, the QF-PCR reaction amplifies a 103 bp sequence from X chromosome and a 109 bp sequence from chromosome 3.

The T3 marker is also a non-polymorphic X chromosome counting marker that is used to determine the number of X chromosome comparing with chromosome 3 by using identical primers. For this marker, the PCR reaction amplifies a 148 bp sequence on X chromosome and a 144 bp sequence on chromosome 3. In a normal female, X chromosome counting marker an area ratio of 2:2 is expected. In normal males and females with monosomy of X a 2:1 ratio is expected. Our result showed that the T3 marker produced a peak area ratio of 2:2 indications the presence of two X chromosomes.

The markers sY84, sY86, sY127, sY134, sY254, sY255, sY1191, sY1192, sY1291 and SRY are non-polymorphic STR markers present on Y chromosome only. It therefore should produce single peak for males who has only one Y chromosome. Since the chromosomal location and the primer binding sites are different for these markers, it is highly unlikely that they will have same number of repeats from both the chromosomes for all these markers. In our patient, we found not only a SRY negative peak but also other negative peaks for specific markers on Y chromosome. Moreover, the co-amplification of DAZ/DAZL and CDY2/CDY1 fragments that permits determination of the DAZ, CDY1 and CDY2 gene copy number. The DAZ gene copy number was quantified using primers that co-amplify a fragment of intron 10 from DAZ gene (211 bp) and from the homologous autosomal locus DAZL on chromosome 3 (214 bp). The relative ratio of the two CDY1 genes in the AZFc region and two CDY2 genes in the AZFb region, which share 98% nucleotide identity which amplify a 6 bp nucleotide difference in the S’ region, producing fragments of 204 bp for CDY1 and 198 bp for CDY2. There were no peaks for any DAZ/DAZL and CDY2/CDY1 fragments in QF-PCR result. In conclusion, we obtained no peaks from all the markers on Y chromosome for the patient. This indicates the absence of this chromosome in the patient (Figure 1).

Repeat peripheral blood cultures from this phenotypically normal male showed a normal female chromosome complement with 46, XX.

Considering the findings described above it can be concluded that the tested individual was having SRY-negative 46, XX male.

Discussion

The reported incidence with 46, XX karyotype are sterile with normal female chromosomes varies from 1 in 9,000 to 1 in 20,000 in newborn males [27,28]. Most cases are just sporadic, without familial clustering [6]. The number of the 46, XX males are known to carry variable amount of Y chromosome material containing the SRY region due to an XY chromosomal interchange during paternal meiosis, leading to the differentiation of primitive gonad into testes is 90%. The majority of cases recorded are due to interchange of a fragment of the short arm of the Y chromosome containing the region that encodes the testes determining factor (TDF) with the X chromosome [19,29-31]. Others include mutation in an autosomal or X chromosomal gene, which permits testicular determination in the absence of TDF, and undetected mosaicism with a Y-bearing cell line [32]. Situated in the short arm of Y chromosome (Yp), the SRY is the main testes determining factor, and its presence drives the bipotential primitive gonad towards testes development [33]. It is shown that SRY and other Y-chromosome regions was negative in peripheral blood DNA. Three azoosperma factor regions (AZFa, AZFb and AZFc) in Yq is the location that genes related to spermatogenesis to be harboured. Microdeletions occurring in the long arm of the Y chromosome in multiple genes and are denoted as AZFa, AZFb, and AZFc are found in 10–15% of men with azoospermia or severe oligospermia [7,34]. Deletions are fertility [6] in these different regions having varied effect on spermatogenesis. The overall AZF region is essential for spermatogenesis [7]. Absence of these regions on Yq explains the universal presence of azoosperma, despite testes being the gonad present in these patients. The majority of cases have normal external genitalia, but 10% to 15% of XX males show various degrees of hypospadias [35]. Molecular studies have detected Y chromosome material in 75% of XX males, which explains their testicular development [30]. Testicular histology usually reveals complete Sertoli cell-only syndrome and hyperplasia of the Leydig cells. On the basis of karyotype analysis and detection of SRY gene, 46, XX male patients can be clinically 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 [18]. In an opposite way, shortly after birth, the abnormality of genitalia could be easily found in SRY gene-negative patients; some patients even show genital ambiguity. Moreover, masculinity signs are not clear in SRY gene-negative patients; especially in adult patients: breast development and the female secondary sex characteristics can be found [18]. In our studied case, on the contrary the literature [18], masculinity signs were normal: his breast development and the male secondary sex characteristics were as usual, and also SRY gene-negative. When comparing with Mustafa et al in 2010 we had the same result. 46, XX male sex reversal syndrome has different clinical symptoms, and its pathogenesis may involve many different mechanisms from the perspective of genetics, clinical, karyotype analysis and related routine checks can only provide preliminary information, but cannot provide more treatment and prognosis.

Conclusion

In this study, a SRY-negative 46, XX male has fully mature normal
male genitalia with infertility. Analysis of 30 metaphases showed only 46, XX cell populations without any numerical or structural chromosomal aberrations. The elevated gonadotropins place the site of the lesion at the testicular level, and the chromosomal analysis confirms the diagnosis.

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References