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A CASE OF 46,XX TESTICULAR DISORDER OF SEX DEVELOPMENT: CLINICAL, MOLECULAR AND CYTOGENETIC STUDIES

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Abstract

Aim. To investigate the cause of infertility in an azoospermic man and to describe the phenotype of a new 46,XX male case.

Case report. We present the case of an infertile man, 33 years old, with a history of several years of infertility, diagnosed with the 46,XX male syndrome, SRY positive. The patient was diagnosed by clinical, hormonal, ultrasound and genetic criteria. Our patient was born at 39 weeks of pregnancy, from unrelated parents. The mother's age was 22 years old and father's age was 23 years old at the time of the conception. Both of his parents were exposed to chemical noxae before his conception. The case we report is a SRY positive 46,XX male with complete masculinization, confirmed by FISH and molecular analyses, caused by an X/Y chromosome inter-change during paternal meiosis.

Conclusions. In our case, the SRY translocation, could probably be related to the paternal exposure to external factors like chemical noxae, but more data are necessary. Cytogenetic and molecular analyses are necessary for an accurate diagnosis, as well as endocrine testing and pelvis ultrasound.

Key words: 46,XX testicular DSD, infertility, SRY gene, molecular analyses, FISH.

INTRODUCTION

The 46,XX testicular disorder of sex development (DSD), previously termed "XX male syndrome", is a rare abnormality defined by the discordance between the phenotype and the genotype of an individual, with complex pathogenic mechanisms. It is a part of the large group of disorders of sexual differentiation.

The new nomenclature for XX male syndrome is 46,XX testicular DSD, according to the International Consensus Conference on the Management of Intersexuality held in October 2005, under the auspices of the Lawson Wilkins Pediatric Endocrine Society (USA) and the European Society for Pediatric Endocrinology.

The first case with male phenotype but with 46,XX karyotype was reported 50 years ago, in 1964 by De La Chapelle (1). Until now almost 300 cases have been described.

Individuals with male phenotype but 46,XX karyotype appear about once in 20000 births (2 - 4).

More than 100 cases characterized by a male phenotype with 46,XX karyotype have been described since 1996.

About 80-90% of these patients present a spontaneous translocation of the SRY gene from Y to X chromosome. Some cases are SRY negative, determined by the duplication of SOX 9, 22 q or R SPO 1 defects or gonadal mosaicism (5-8).

Generally, most of the patients are diagnosed in the adulthood, when they are investigated for infertility. This is such a case we present. Other patients are diagnosed sooner because they present various degrees of sexual ambiguity or hermaphroditism (5, 9).

CASE REPORT

We present the case of an infertile man, 33 years old, with a history of several years of reproductive failure, who was investigated for infertility in our Andrology department.

The patient gave his informal consent for the medical procedures.

Clinical and andrological examination, hormonal and cytogenetic evaluations, as well as sperm analyses were performed.

Our patient was born at 39 weeks of pregnancy, from unrelated parents. The mother's age was 22 years old and father's age was 23 years old at the time of the conception. Both of his parents were exposed to

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chemical noxae before his conception. He has a healthy older sister.

The physical examination revealed a short stature for a male (mother's height is 175 cm and father's height is 182 cm) with the height of 169 cm (-1.3 SD), which is 2.14 SD below his target height (184.6 cm), with normal body proportions, a weight of 102 kg (BMI = 33.3 kg/m^2), arm span: 166 cm, pubis – vertex distance: 82 cm, heel - pubis distance: 87 cm, chest circumference: 102 cm, waist circumference: 115 cm, a protuberal abdomen with no stria. The adipose tissue had a typical female distribution and bilateral gynecomastia was present.

Axillary and pubic hair were of normal pattern. Examination of the genitalia revealed a small scrotal sack with hypoplastic testes (volume of around 2.5 ml each), normally located, a small penis (5 cm) with a normal urethral opening. Our patient had a normal sexual life, without any sexual dysfunctions, normal psycho-sexual identity and intelligence (Table 1).

The patient's phenotype is presented in Figs. 1, 2.

Hormonal laboratory evaluation revealed hypergonadotropic hypogonadism. The hormonal profile was abnormal with elevated serum concentrations of gonadotrophs and very low levels of inhibine B. Free testosterone, prolactin, serum cortisol, TSH, 17 hydroxy progesterone, are within the normal reference range.

The levels of the analyzed hormones are presented in Table 2.

Table 1. Clinical findings of the patient

Age	33
Height (cm)	169
Body weight (kg)	102
Body mass index (BMI – kg/m^2)	33,3
Arm span (cm)	166
Vertex – pubis distance (cm)	82
Heel – pubis distance (cm)	87
Chest circumference (cm)	102
Waist circumference (cm)	115
Adipose tissue	Female distribution
Gynecomastia	+ bilateral
Axillary and pubic hair	Normal pattern
Facial hair	Normal
Penile length (cm)	5
Scrotal sack	Small
Palpable bilateral vas deferens	+
Testicular volume (mL)	2.5 each
Libido	Normal
Erection	Normal
Semen analysis	Azoospermia
Hormonal profile	Hypergonadotropic hypogonadism

Semen analyses, which were done three times, revealed azoospermia.

Chromosomal analyses performed on the peripheral blood lymphocytes, revealed 46,XX karyotype. G banded chromosomes from cultured peripheral blood were analyzed with conventional methods. Chromosomes preparations were carried out according to standard techniques. Twenty metaphases were counted (Fig. 3).

There was no evidence of mosaicism. SRY was identified by Polymerase Chain Reaction – PCR (Fig. 4).

The genomic DNA was obtained from peripheral blood leukocytes using a commercially available kit (Quick-gDNA Blood MiniPrep, ZymoResearch, CA, USA).

The AZF microdeletions, situated on the long arm of the Y chromosome, were assessed by multiplex PCR assays. The initial screening was performed by analyzing the set of 6 STS (sequence-tagged sites) markers recommended by the EAA/EMQN guidelines (sY86 and sY84 – AZFa region; sY127 and sY134 – AZFb region; sY254 and sY255 – AZFc region), plus the SRY and ZFY genes (10). Subsequently, a more in depth analysis was performed. This comprised 20 STS markers from the AZF regions, analyzed by a multiplex PCR assay, using a commercially available kit, according to the manufacturer's instructions (Y Chromosome Deletion Detection System, version 2.0, Promega, Me, USA). This kit covers the following markers: sY14 (the SRY gene), sY81 (proximal to the

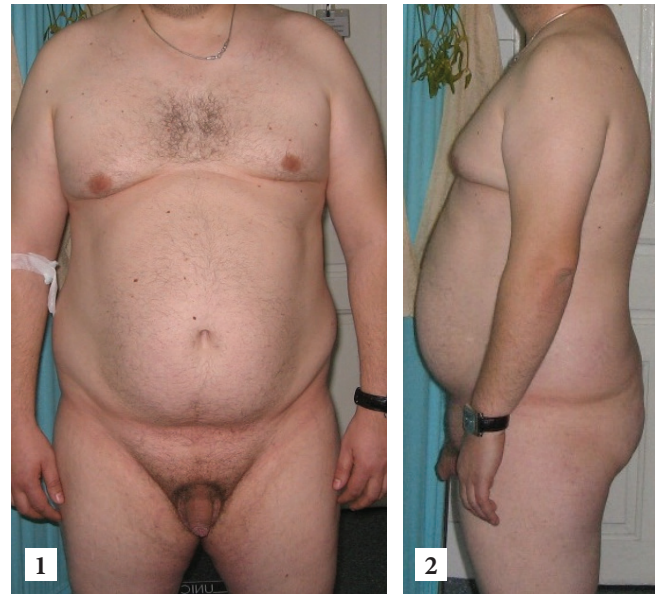

Figure 1. Patient's phenotype. Clinical aspect: microorchidia, small penis, bilateral gynecomastia. **Figure 2.** Clinical aspect: small scrotum, protuberant abdomen with no stria.

Table 2. Hormonal profile of the patient

Hormones	Patient's value	Normal range
FSH	31.1 mUI/mL	0.7-11.1 mUI/mL
LH	14.2 mUI/mL	0.8-7.6 mUI/mL
Free testosterone	16.09 pg/mL	4.5-42 pg/mL
Total testosterone	13.2 nmol/L	9.9-27.8 nmol/L
Inhibine B	< 10 pg/mL	60-325 pg/mL
PRL	2.71 ng/mL	2.5-17 ng/mL
Estradiol	31 pg/mL	< 20-47 pg/mL
DHEA-S	269.7 mcg/dL	104-464 mcg/dL
Cortisol	9.24 mcg/dL	6.7-22.6 mcg/dL
TSH	1.49 µUI/mL	0.5-4.5 µUI/mL
17 hydroxiprogesterone	1.42 ng/mL	0.3 – 3 ng/mL

AZFa region), sY86 and sY84 (AZFa region); sY182 (the KALY gene); sY121, sYPR3, sY124, sY127, sY128, sY130, sY133, sY134 (AZFb region); sY145, sY152, sY242, sY208, sY254, sY255 and sY157 (AZFc region).

The molecular analysis revealed the absence of all STS markers situated in the AZF region. However, the SRY and ZFY genes were present.

FISH studies with a probe containing SRY gene were also performed following the standard protocol (Fig. 5).

FISH for SRY gene - 200 FISH-metaphases and 30 interphases assays were performed. Probes used: XL SRY (for SRY gene –red signal) and DXZ1 (for chromosome X centromere –blue signal).

The tested metaphases showed a red signal on one of the X chromosomes, corresponding to the SRY gene. X chromosomes were recognized in the 2 blue signals sent by probes DXZ1. Two blue signals were also detected in the interphases (corresponding to the two X chromosomes)

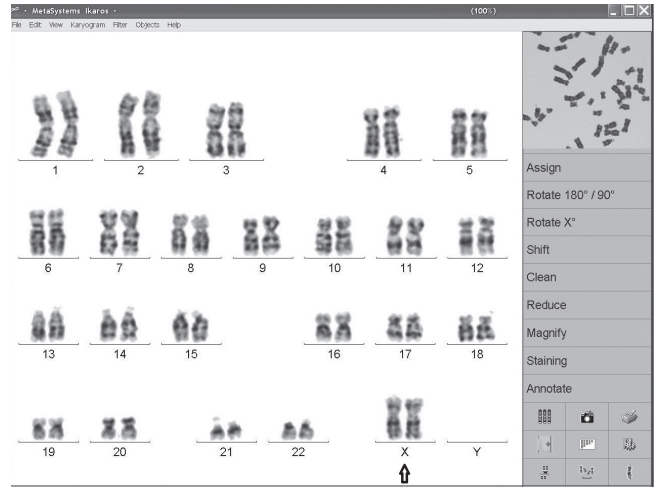

Figure 3. Karyotype analysis of patient chromosomes: GTG banded metaphase chromosomes (550 level bands) are shown 46,XX – pointed by the arrow.

and a red signal, corresponding to SRY gene.

The cytogenetic formula is: 46,XX.ish der (X) t(X;Y)(p22.3;p11.3)(SRY+,DXZ+)nuc ish (DXZx2, SRYx1) - SRY gene is translocated on one of the X chromosomes.

Testicular biopsy was not carried out. The ultrasound of the pelvis showed a normal sized prostate, no evidence of uterus or ovaries (Fig. 6).

Testicular ultrasound revealed testes hypoplasia (Fig. 7).

DISCUSSION

Human sex development is a complex process. Male sex differentiation requires the interaction among multiple genes including : SRY, SOX 9, DAX-1, SF-

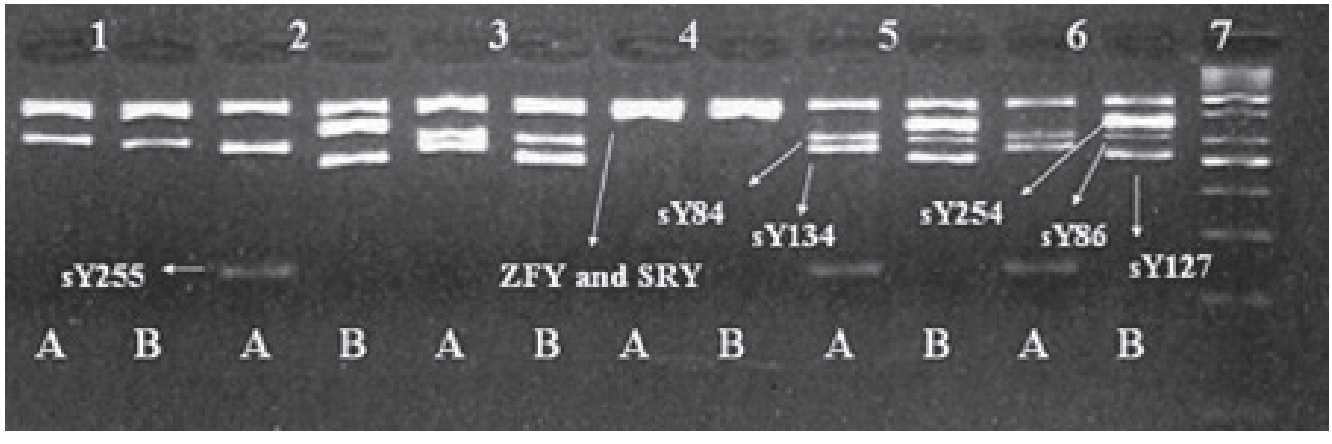

Figure 4. PCR identification of SRY – in each patient, two multiplex reactions are used: A, which amplifies ZFY, SRY, sY84(AZFa), sY134(AZFb) and sY255(AZFc) and B, which amplifies ZFY, SRY, sY254(AZFc), sY86(AZFa) and sY127(AZFb). 3% agarose gel showing:1 – patient with AZFb+c deletion (absence of sY127, sY134, sY254 and sY255); 2 – patient with AZFa deletion (absence of sY84 and sY86); 3 – patient with AZFc deletion (absence of sY254 and sY255); 46,XX patient showing only ZFY and SRY and the absence of all AZF markers; 5,6 – men without AZF deletions; 7 – 50 bp molecular weight DNA marker.

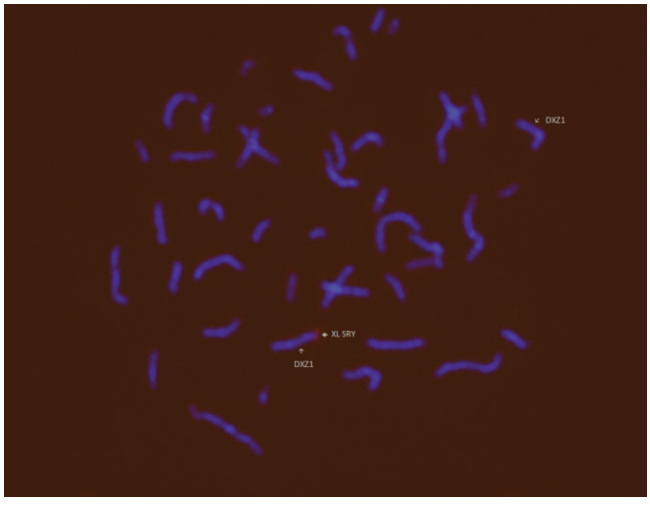

Figure 5. Fluorescent in situ hybridization (FISH) on metaphase chromosomes with the XL SRY(red)/CEP X (blue) probes(Metasystem). Metaphase spread showing a normal X chromosome (blue signal for centromeric DXZ1 locus) and the SRY(red)translocates to the distal end of short arm of chromosome X.

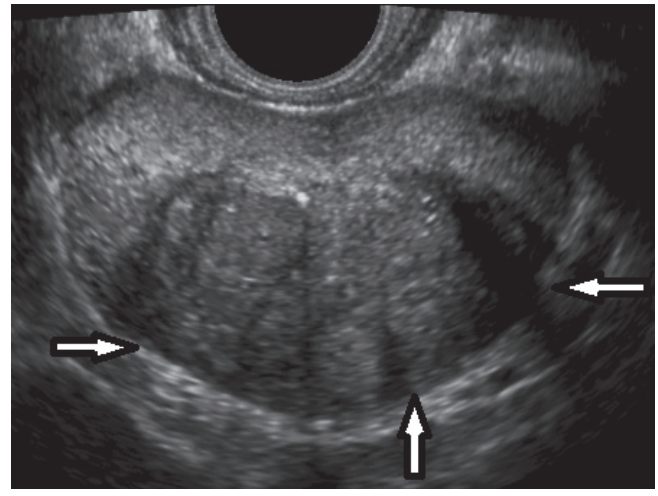

Figure 6. Pelvis ultrasound shows the prostate normal in size – pointed by the arrows.

1, AMH, FGF9, DMRT1. The process of male sex determination still has a lot of unknown aspects, but there is consensus that genes of the Y chromosome, in particular sex determining regions Y gene (SRY), plays a major role (11,12).

In XY embryo SRY induces the gonadal primordium to develop into testis (13) and SOX 9 gene has been shown to be involved in sex determination pathway downstream to SRY gene.

The SRY gene is located on the short arm of the Y chromosome, from base pair 2.786.854 to base pair 2.787.740. SRY activates the transcription of the SOX 9, which in its turn activates the FGF 9 gene, necessary to the Sertoli cells differentiation. Through

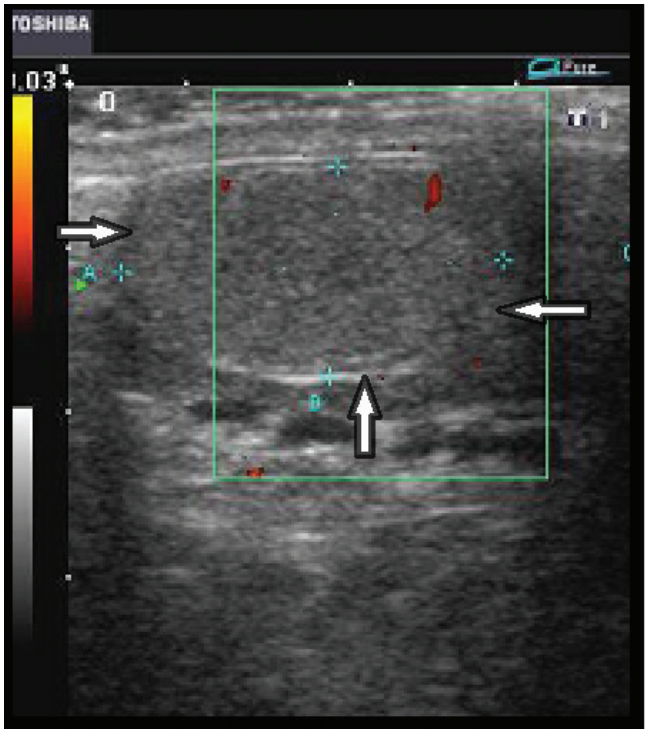

Figure 7. Scrotal ultrasound showing testes hypoplasia – pointed by the arrows.

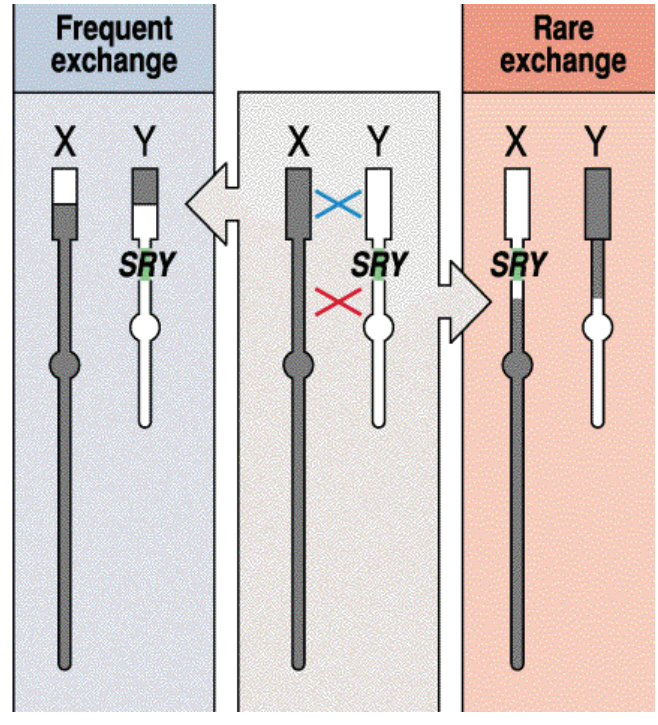

Figure 8. Chromosome X SRY.

the proper activation of the SOX 9 gene, the bipotent cells of the primitive gonads, begin to differentiate in Sertoli cells, allowing the appearance of the primordial testis.

SRY gene translocation, SRY gene mutations or the absence of SRY are associated with the disorders of sexual differentiation. In the absence of SRY, the development of the male phenotype probably results from the loss of function mutation in some unknown sex determining genes (which normally inhibits the male pathway) or from a gain of function mutation in a gene downstream to SRY in male pathway (14).

Several theories (such as the autosomal gene theory, the mosaicism theory) tried to explain the etiology of 46,XX testicular DSD. A mouse model for this condition (XX male) has been studied in which mice have two X chromosomes and testis, because a fragment of the Y chromosome is translocated on to one of the X chromosomes. SOX 9, like SRY, were reported to be adequate to induce testis differentiation in transgenic XX mice (15). In 80-90% of 46,XX males, SRY gene is present as a consequence of a translocation from Y to X chromosome, during paternal meiosis, but 46,XX males with no evidence of Y specific DNA have also been reported – Fig. 8 (16).

Testicular development occurs even in the absence of the SRY gene and such cases are probably explained by up-regulating duplication and mutation of SOX 9 gene (5). Not only SOX 9 duplication was reported to cause testes development in the absence of SRY gene, but also DAX 1 (Sutton E. *et al.*, 2011, Moalem S. *et al.*, 2013) and recently SOX 3 and FGF 9 duplications (17).

Cytogenetic and molecular analyses (SRY sequencing) are necessary for an accurate diagnosis (18), helping the clinicians to classify the XX maleness into 3 subgroups: a) 46,XX males SRY positive; b) 46,XX males without SRY gene and c) 46,XX/46 XY mosaicism (4).

A great variability of the phenotypes was described in 46,XX testicular DSD. All the adults with this condition are infertile and have small testes (19, 20). Cryptorchidism is present in 18% of cases and hypospadias in 10-15% of patients. Bilateral gynecomastia and short stature are often present. Less than 20% of cases are diagnosed before adolescence, because of uro-genital malformations. There are two categories of 46,XX testicular DSD: the first is the classic XX male with almost normal phenotype and the second one is the non classic XX male individuals with sexual ambiguity and XX true hermaphrodites. The great variability of phenotypes seen in SRY positive 46,XX testicular DSD may be explained by two mechanisms: X chromosome inactivation pattern and the amount of X material including SRY gene that has been translocated to the X chromosome (21). The phenotypic differences observed in SRY positive 46,XX testicular DSD are dependent on the proximity of the breakpoint to the SRY gene, as well as the presence or absence of cryptic rearrangements affecting the expression of the SRY gene (21).

Our patient is from the first category (the classic XX male with almost normal phenotype). He has a short stature for a male, with normal body proportions, he has microorchidia, bilateral gynecomastia, small penis, normal male psycho-sexual identification and intelligence, normal secondary sexual characteristics.

The short stature may be explained by loss of putative Y chromosome growth controlling genes or epigenetic consequences of SRY presence as XIST hypomethylation though it was shown that SHOX is normally expressed (Poplinski A. *et al.*,2010), random inactivation of short and long AR alleles in 46,XX testicular DSD patients, comparable with normal females (22).

Hormonal laboratory evaluation revealed hypergonadotropic hypogonadism. Until now none of the reported cases were connected with external factors like toxins, paternal trauma or infections, but in our case the father's patient was exposed to chemical noxae (heavy metals) and radioactive elements.

Ferrer T. *et al.* described 5 SRY negative 46,XX testicular DSD patients. Three of them were XX male individuals with normal immature testis on biopsy and the other two were true hermaphrodites. The authors postulated a theory for male sex determination including a downstream gene on the X chromosome in which expression is influenced by X inactivation.

Phenotypic variations in two siblings with paternally derived SRY bearing X chromosome has been described by Abbas N. (23). One of the brothers was a phenotypic male and the other was a true hermaphrodite. Rajender S. (2006) reported a case of SRY negative XX male with complete masculinization, with no evidence of SOX 9 and DAX 1 genes mutation or any deletion on the X chromosome (14).

Breast carcinoma and testis neoplasia have been reported in association with complete sex reversal SRY positive 46,XX testicular DSD, and for this reason patients with this condition need lifelong monitoring (20, 24).

From Romania there have been also reported some other cases by Procopiuc (2009), Pepene (2008).

Grigorescu-Sido *et al.* (2004) reported three new 46,XX males and their correlation between genotype (SRY +/-) and phenotype (9). Two patients were phenotypically normal XX male (10 and 13.5 years old) SRY positive and one of 3.1 years old with ambiguous external male genitalia Prader IV, was 46,XX karyotype without SRY, according to FISH and molecular analyses. The authors concluded that the presence of the SRY gene results in a more masculinized phenotype, but other studies did not confirm it (14 ,16).

However, data from the literature do suggest that the majority of SRY negative 46,XX testicular DSD patients have a variable degree of ambiguous genitalia in contrast to SRY positive patients. Despite the fact that SRY gene is the main regulatory factor for testis determination, phenotypic variability showed in 46,XX testicular DSD cannot be explained only by whether SRY gene is present. Other genes such as SOX 9, DAX 1, WT 1, FGF 9 have been involved in the process of gonadal differentiations (21).

Our patient, like the majority of 46 XX males reported by other authors, was SRY positive, caused by an X/Y chromosomal inter-change during paternal meiosis, leading to the differentiation of primary gonads into testis. In a study recently published in March 2013, from 11 males with 46,XX karyotype, only a single patient was SRY negative (13).

Endocrine data revealed that their endocrine hormone levels were similar to those observed for Klinefelter syndrome with higher FSH and LH levels and lower testosterone levels, but our patient had normal total testosterone and free testosterone levels. The hormonal replacement therapy is necessary in the presence of hypergonadotropic hypogonadism.

Paternal karyotype and FISH for SRY could be performed to determine whether or not the father carries a balanced translocation (Xp:Yp) or if the translocation occurred in the germline, or *de novo* (25).

In conclusion, in our case, the SRY translocation, could probably be related to the paternal exposure to external factors like chemical noxae, but more data are necessary. For the complete assessment of these patients, Y DNA genomic analysis, endocrinological testing, pelvis ultrasound and gonadal biopsy are recommended.

Psychological counseling is necessary in order to help the patients to accept this medical condition (and its consequence such as infertility).

The etiology of 46,XX testicular DSD still has a lot of unelucidated aspects and more researches are necessary.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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