

A Rare Case of Sry-Negative 46, XX Testicular Disorder of Sex Development with Complete Masculinization: A Case Report and Literature Review

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Abstract

46, XX testicular disorder of sex development is a rare cause of male infertility. There is a disagreement between the manifested sexual phenotype (male) and the genotype (female). It occurs in males with 46, XX karyotype who may have normal or ambiguous male genitalia, testosterone deficiency and infertility. With adequate hormonal treatment, the chance of having a good quality of life is high. In the diagnosis, the genetic study is fundamental for the identification of the gene that causes the disorder. The role of SRY gene is important because it allows classifying these males into two groups: those with SRY gene, which activates male sexual differentiation, translocated from Y chromosome to X (SRY-positive), and those without this translocation (SRY-negative). 80-90% of cases are SRY-positive. In the 10-20% SRY-negative it is difficult to identify the gene responsible for the alteration. This would indicate the involvement of other genes linked to the X chromosome. This is a review of the first case of SRY-negative 46, XX testicular disorder of sex development with complete masculinization reported in Basque Country in which the cause of the disorder is being investigated, even without specifying.

Keywords: 46, XX karyotype; Azoospermia; Disorder of sex development; Hypergonadotropic hypogonadism; SRY-negative; XX male

Abbreviations: DSD: Disorder of Sex Development; SRY: Sex-Determining Region Y; AZF: Azoospermia Factor; FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone; FISH: Fluorescence *in Situ* Hybridization

Introduction

46, XX testicular DSD is a rare cause of male infertility. It is characterized by a lack of correlation between the manifested sexual phenotype (male) and the genotype (female). These people, phenotypically males, have 46, XX karyotype, male external genitalia ranging from normal to ambiguous, two testes, absence of Müllerian structures and testosterone deficiency, in addition to azoospermia that results in infertility [1-3]. The process of sexual differentiation in humans is not completely known, but the implication of SRY gene is clear: it has a crucial role in this differentiation [4]. It is normally located on the distal region of the short arm of the Y chromosome (Yp11.32) and induces the testicular development of the undifferentiated gonad, from the 4th week of embryonic development. From the 4th month, the fetal gonad produces anti-müllerian hormone that induces male differentiation of the external genitalia. The genes involved in spermatogenesis, AZF, are located on the long arm of the Y chromosome (Yq) [5].

At a molecular level, patients with 46, XX testicular DSD are divided into two groups according to the presence or absence of the SRY gene: SRY-positive or SRY-negative. The SRY gene is found in the X chromosome in 80-90% of male patients with XX karyotype without sexual ambiguity. The presence of a Y chromosome fragment on the short arm of the X chromosome is explained by an unequal exchange between the homologous regions of the short arms of the X and Y chromosomes during the paternal meiotic division [1-3]. The presence of SRY is important because it determines the differentiation of the testicle and the consequent masculinization of the individual; however, completely masculinized patients have been described in the absence of detectable SRY. This could be interpreted as a hint that, although

the action of SRY gene is necessary for the testicle to differentiate and produce a male phenotype, the presence of other genes may have the same effect. The authors report a case of SRY-negative 46, XX testicular disorder of sex development. The cause of the disease in this case has been studied by looking for abnormalities in other genes different from SRY gene, although it has not yet been possible to specify. This is the first case of SRY-negative 46, XX testicular DSD reported in Basque Country. Because of the rarity of this disorder, we consider interesting its review through the study of this case.

Case Presentation

The patient of our study was a 13-year-old boy. He consulted in pediatric endocrinology for the control of puberty and height. Background: He was born with 50 cm and 2,800 gr. He was operated 4 times because of important hypospadias. His parents and older sister were healthy: the father's height was 176.3 cm and the mother's was 157.9 cm. Physical examination revealed a male phenotype, without dysmorphism, height 153.7 cm, weight 63.350 kg, broad thorax, short extremities, penis and testicles of normal size, and female voice. Examinations of bone age were requested, revealing a bone age of 14 years.

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Analytcs

The results of the elementary biochemical and hemogram parameters were normal. The hormonal study revealed: FSH 31.9 U/L [1.7-11], LH 12.2 U/L [0.5-6] and total testosterone 2.08 ng/mL [2.4-10.7]. In several subsequent analytical determinations, the gonadotropin elevation was confirmed and testosterone remained at levels close to the lower limits of normality (FSH 42.8 U/L, LH 21.7 U/L, total testosterone 2.22 ng/mL).

Genetic study

Given the results of hypergonadotropic hypogonadism, a cytogenetic study was carried out, performing molecular karyotyping in peripheral blood. The analysis showed a chromosomal formula of 46, XX. After carrying out FISH, the absence of SRY gene was observed. There was a suspicion of SRY-negative 46, XX testicular DSD. The patient and the family were informed of the genetic alteration and he started the testosterone replacement treatment with testosterone cypionate 50 mg/month. Months later the dose was increased to 100 mg/month. In order to determine the origin of the disease, a molecular study of the *SOX9* gene was performed. The aim of this study was to analyze the presence of large deletions or duplications of this gene, which could be associated with the 46, XX testicular DSD. The patient did not present abnormalities in the analyzed regions of the *SOX9* gene. Therefore, the pathology is due to another cause not yet determined. At 18 years old the patient was referred to Adult Endocrinology. He was currently undergoing treatment with testosterone cypionate 250 mg every 3 weeks and in control with analytes for the measurement of serum testosterone concentration at a three-month interval. With testosterone replacement therapy, the levels of FSH, LH and total and free testosterone remained close to normal for men, without symptoms of hypogonadism.

Discussion

46, XX testicular disorder of sex development is a rare disease that occurs in men with 46, XX karyotype. There is a disagreement between chromosomal sex (female), and gonadal sex and phenotype (male). It affects 1/20,000 men and represents 2% of cases of male infertility. It is not known if any population has a higher or lower risk of suffering this disorder [2,6]. It is also known as “De la Chapelle syndrome” because it was described by Albert de la Chapelle in 1964 [2,7]. Until a few years ago, it was also called “XX male syndrome”, but at an international consensus conference on the management of intersexuality organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society of Pediatric Endocrinology, a multidisciplinary group of experts proposed the name “XX male syndrome” to be replaced by “46, XX testicular disorder of sex development” [8]. This disorder is characterized by the presence of 46, XX karyotype, male external genitalia ranging from normal to ambiguous, two testes, azoospermia, absence of Müllerian structures and testosterone deficiency. Classically, three groups have been described according to the phenotype: XX males with normal male internal and external genitalia; XX males with sexual ambiguity, frequently detected at birth, although they are sometimes diagnosed in adulthood by ambiguous external genitalia; and XX true hermaphrodites, with ambiguity of internal or external genitalia detected at birth. Most of the patients present, after puberty, male phenotype, normal pubic hair and normal penis size, small testes and infertility resulting from azoospermia. They can also show gynecomastia, short stature, obesity, cryptorchidism and hypospadias. Referring to the gender identity, they are considered as men. Approximately 15% of individuals have ambiguous genitalia at

birth. The natural history of these individuals, if they are not treated, is similar to the typical consequences of testosterone deficiency: low libido and possible erectile dysfunction, decreased secondary sexual characteristics, such as poor body hair, little need to shave and decreased muscle mass and strength, increased fat mass and increased risk of osteopenia and depression [1,2].

Patients can be divided into two categories: those who have variable amounts of Y chromosome sequences, and those who do not. Carriers of sequences of the Y chromosome have the SRY gene translocated from the Y chromosome to the short arm of the X chromosome. The SRY gene encodes the so-called determinant factor of testicular differentiation, whose presence and expression are necessary to inactivate the signals of female sexual differentiation and activate male sexual differentiation. 80-90% of cases present a translocation of the SRY gene and are considered SRY-positive. Normally, the development of genitalia and male secondary sex characteristics is normal, despite the fact that they present azoospermia. Although initially the presence of SRY gene was associated to normal male genitalia and its absence to ambiguous genitalia, an increasing number of published cases describe SRY-negative 46, XX patients (10-20% of cases) with male phenotype, which would indicate the implication of other genes linked to the X chromosome as the cause of the disorder: *SOX9*, *SOX3*, *SOX10*, *RSOY1*, etc. [9,10]. The diagnosis is established by combining clinical findings, endocrinological study and cytogenetic analysis showing the clinical characteristics mentioned above, hypergonadotropic hypogonadism, with high gonadotropin hormones and low testosterone, and 46, XX karyotype. Incongruence between the chromosomal sex, the gonadal sex and the phenotype is identified. FISH analysis determines the presence or absence of the SRY gene.

The main differential diagnoses are 45,X0/46,XY or 46, XX/46,XY mixed gonadal dysgenesis, 47,XXY Klinefelter syndrome, 46, XX ovotesticular DSD and sex chromosome mosaicisms. In this review, the case of a “normal” male with male secondary sexual characteristics, normal height and intellectual development, mature genitalia and no skeletal anomalies is described. However, after a deeper examination, hypergonadotropic hypogonadism (elevated FSH and LH and decreased testosterone), SRY-negative 46, XX karyotype and azoospermia are observed.

The causes of testicular tissue induction in SRY-negative 46, XX patients remains unknown. There are different theories:

- SRY-positive hidden gonadal mosaicism [11].
- Presence of a marker chromosome in XX males or true hermaphrodites [12].
- SRY gene that acts by inhibiting an autosomal recessive gene, called “Z”, whose protein normally inhibits male development. The homozygous mutation produces male phenotype and the heterozygous mutation ambiguous genitalia or hermaphrodites [4].
- *SOX9* gene located on chromosome 17q24. Male development is usually originated by the expression of the SRY gene, which initiates a cascade of gene interaction, orchestrated by the *SOX9* gene, which produces the differentiation of the immature gonad into testicles. *SOX9* is capable of causing the development of the testes in the absence of the SRY gene by ectopic expression or overexpression. It has also been seen that duplications or deletions in the regulatory region of the *SOX9* gene cause its expression to increase or decrease leading to one or another alteration. However, there are cases where a

duplication upstream of *SOX9* is not positively correlated with the SRY-negative 46, XX testicular disorder of sex development [13,14].

- *SOX3* gene located on chromosome Xq27.1. It has been suggested that the *SOX3* and *SRY* genes are functionally interchangeable in sexual determination since both are part of a regulatory pathway that leads to the differentiation of testicles from the immature gonad [15].
- *SOX10* gene located on chromosome 22q13.1. A case of an individual, 46, XX karyotype with male phenotype, who had a duplication in 22q that includes the *SOX10* gene has been presented. There are no other studies that confirm that 22q/*SOX10* duplication causes males 46, XX [16].
- *RSPO1* gene located on chromosome 1p34.3. It has been seen that this gene causes sexual reversion encompassed within palmoplantar hyperkeratosis and skin squamous cell carcinoma syndrome [17].
- Other genes: *FGG9* gene located on chromosome 13q12.11; *SPRY2* gene located on chromosome 13q31.1; etc.

In the case described, several genetic studies have been carried out to determine the cause of the disorder. The implication of the *SOX9* gene has been analyzed, but no anomalies have been observed. Therefore, the cause is still unspecified. *SRY*-positive 46, XX testicular *DSD* is usually not inherited, as it results from abnormal *de novo* exchange between Y and X chromosome, with the presence of *SRY* on the X chromosome and infertility. When *SRY* is translocated to another chromosome or when fertility is preserved, an autosomal dominant inheritance limited by sex is observed. The mode of inheritance of *SRY*-negative 46, XX testicular *DSD* is not known, but autosomal recessive inheritance has been suggested. 46, XX testicular *DSD* related to *SOX9* is inherited in an autosomal dominant manner, but the inheritance of 46, XX testicular *DSD* related to *SOX3* is not known. The treatment consists in the progressive administration of testosterone to avoid the consequences of the hormonal deficiency [2]. It is always necessary an adequate genetic counseling, as well as psychological assessment in patients who need it. Sensitivity is important when transmitting information about the genetic cause and associated infertility to these individuals. This information should be presented in a way that helps minimize psychological distress. With an adequate hormonal treatment, the chance of carrying a good quality of life is high.

Conclusion

The authors report a rare case of *SRY*-negative 46, XX *DSD* diagnosed in a young man and it is the first case reported in Basque Country with these characteristics. The diagnosis is established by combining clinical findings, endocrinological study and cytogenetic analysis. The genetic study is important to identify the cause of the disorder. In the case presented, several analysis have been carried out, but the gene that causes the disease has not yet been determined. Despite infertility, these patients can lead a good quality of life with a testosterone replacement therapy.

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