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Cytogenetic and molecular characterization of an SRY-negative 46, XX ovotesticular DSD: a case report

Caracterização citogenética e molecular de um DDS ovotesticular 46, XX SRY-negativo: relato de caso

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Abstract

Introduction: *Ovotesticular disorder of sex development is a rare condition characterized by the concomitant presence of testicular and ovarian tissue, and usually presents genital ambiguity. They are chromosomally heterogeneous, and cytogenetic analyses is relevant.* ***Objective:*** *to report a patient from Manaus, Amazonas state, Brazil, with ovotesticular disorder of sex differentiation 46,XX and SRY-negative.* ***Case report:*** *patient aged 19 years, first child of non-consanguineous parents, diagnosed at birth with genital ambiguity and, without correct diagnosis, was registered a male sex. The patient underwent surgery to correct bilateral cryptorchidism, orchiopexy and colpectomy. During puberty, he developed female and male sexual characteristics. Investigation at this time revealed: laboratory (normal total testosterone and estradiol as high follicle-stimulating hormone and luteinizing hormone, histopathological (right gonad, ovarian follicles and left gonad, atrophic testicles), karyotype (46, XX) and molecular (SRY-negative). Diagnosis of ovotesticular disorder of sex development was established. The patient chose to remain male and underwent bilateral mastectomy, vaginal colpectomy and bilateral gonadectomy. Currently, the patient receives hormonal replacement therapy, follow-up with a multi-professional approach and awaits masculinizing genitoplasty.* ***Discussion:*** *For OT-DSD individuals with 46, XX, the female sex is suggested as the best sex of rearing option. Unlike the reported cases, the patient chose the male sex, since the sex at registration of birth was important in his choice.* ***Conclusion:*** *Cytogenetic and molecular analyses allowed us to assist in the etiological diagnosis of the patient with OT-DSD. However, molecular analyses are necessary to elucidate the genes involved in the sexual determination of this patient.*

Keywords: *Genital ambiguity. Chromosome. DSD ovotesticular.*

Resumo

Introdução: *distúrbio da diferenciação do sexo ovotesticular é uma condição rara com presença concomitante de tecido testicular e ovariano, geralmente com ambiguidade genital. Os pacientes são cromossomicamente heterogêneos e a análise citogenética é fundamental.* **Objetivo:** *relatar o caso de um paciente do município de Manaus, Amazonas, portador de distúrbio da diferenciação do sexo ovotesticular 46, XX e SRY-negativo.* **Caso clínico:** *paciente de 19 anos, primeiro filho de pais não consanguíneos, que ao nascimento foi diagnosticado com ambiguidade genital, contudo, sem diagnóstico correto, foi registrado como sendo do sexo masculino. Foi submetido a cirurgias para correção da criptoquirdia bilateral, orquidopexia e colpectomia vaginal***.** *Na puberdade, desenvolveu características sexuais feminina e masculina. Investigação diagnóstica mostrou: exames hormonais (testosterona total e estradiol normais enquanto hormônio folículo-estimulante e hormônio luteinizante elevados), histopatológicos (gônada direita, folículos ovarianos e gônadas esquerda, testículos atróficos), cariótipo (46, XX) e molecular (SRY-negativo). O diagnóstico de distúrbio da diferenciação do sexo ovotesticular foi estabelecido. O paciente optou por permanecer no sexo masculino e submeteu-se à mastectomia bilateral, colpectomia vaginal e gonadectomia bilateral. Atualmente faz reposição hormonal, acompanhamento com abordagem multiprofissional e aguarda pela genitoplastia masculinizante.* **Discussão:** *aos indivíduos DDS-OT com 46, XX é sugerido como a melhor opção de sexo, o feminino. Diferentemente dos casos relatados, o paciente optou por permanecer no sexo masculino, visto que o registro de nascimento foi importante para a sua escolha.* **Conclusão:** *análises citogenéticas e moleculares permitiu auxiliar no diagnóstico etiológico do paciente com DDS-OT, contudo, análises moleculares são necessárias para elucidação de genes envolvidos na determinação sexual desse paciente.*

Palavras-chave*: Ambiguidade genital. Cromossomo. DDS ovotesticular*

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INTRODUCTION

Ovotesticular disorder of sex development (OT-DSD) is a rare condition of sexual differentiation characterized by the concomitant presence of testicular and ovarian tissue, and usually presents with genital ambiguity [1-3]. Approximately 200 cases of ovotesticular disorder of sex development have been reported in the literature, and the molecular pathogenesis remains unclear.

OT-DSD individuals are chromosomally heterogeneous, and may present karyotype 46, XX (70%), mosaicism or chimerism 46, XX / 46, XY (20%) or 46, XY (10%)[4,5]. In most cases, OT-DSD 46, XX has negative SRY (sex-determining region Y), although translocation of the SRY gene from the Y chromosome to the X chromosome may occur [6,7]. Given the above, the objective of this study was to report a patient from Manaus, Amazonas state, Brazil with ovotesticular disorder of sex development with a 46,XX karyotype and SRY-negative.

CASE REPORT

For this report, an informed consent form was given to the patient, and it was duly completed and signed. The patient is 19 years of age, the first child of non-consanguineous parents and without family history of genetic disease. At birth, he was diagnosed with genital ambiguity, which included penoscrotal hypospadias, bilateral cryptorchidism and a micropenis. Although he did not have the correct etiological diagnosis, he was registered as a male.

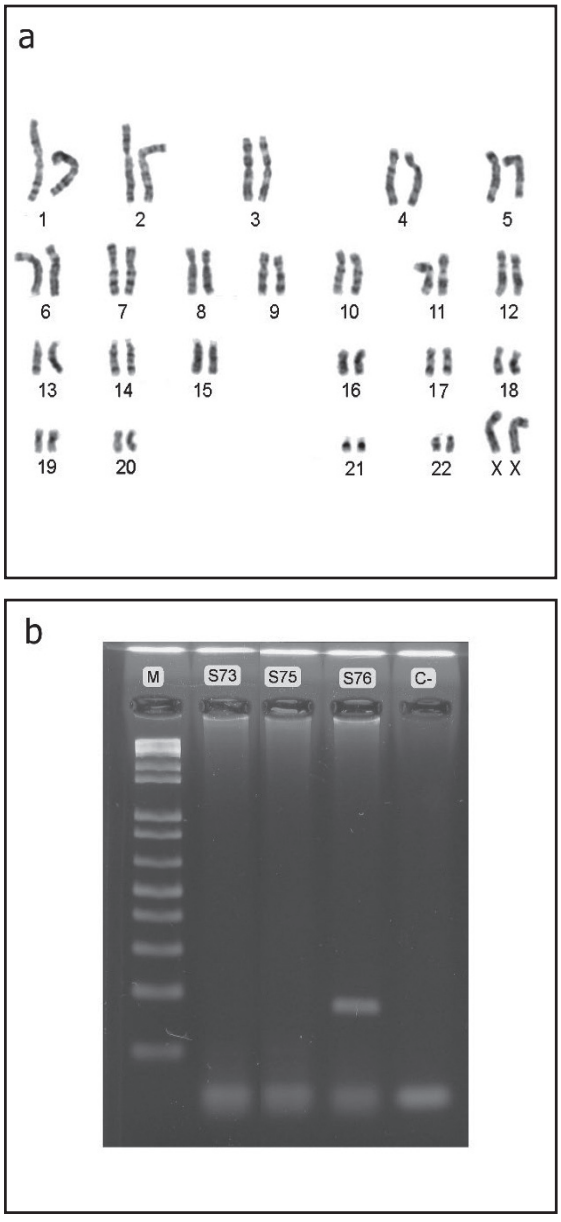
The patient had a surgical history of correction of bilateral orchiopexy. Previous surgeries for the correction of penoscrotal hypospadias were performed, but all without success. He started puberty at the age of 13 with the development of female and male sexual characteristics and was diagnosed late as having an ovotesticular disorder of sex development. On physical examination, a poorly developed phallus, penoscrotal urethra, pigmented/wrinkled scrotal sac and well developed breasts were observed. There was difficulty in palpating the gonads due to the presence of a hydrocele.

Hormone levels were normal for total testosterone 0.90 ng/mL and estradiol 41 pg/ml (male). FSH (29.7 mIU/mL) and LH (9.12 mIU/mL) were outside the normal limits and were considered elevated.

In the histopathological examinations, biopsy of the left gonad identified the presence of seminiferous tubules lined with germinating epithelium consisting of spermatogonia and Sertoli cells. In the stroma, collagenous connective tissue was noted, which was characterized by testicular atrophy. Biopsy of the right gonad revealed ovarian follicles and hydrosalpinx.

The karyotype using G-banding revealed karyotype 46, XX in all 50 cells analyzed (Figure 1a). Molecular polymerase chain reaction (PCR) investigation of the SRY gene was negative (Figure 1b).

Figure 1 – *(a) Karyotype of the patient with DSD 46, XX and (b) agarose gel 2% showing polymerase chain reaction (PCR) using primers specific to the SRY gene. M=marker 1 Kb plus DNA Ladder; S73 = patient reported in this study, SRY-negative; S75 = normal female 46, XX, SRY-negative; S76 = normal male, SRY-positive; C- = negative control.*



Source: Own Authorship

During the diagnostic process, the patient was accompanied by the psychology department, which provided counselling regarding his identity conflict. The patient chose to maintain the social sex of birth registration, i.e., the male sex. He underwent a bilateral mastectomy, colpectomy and bilateral gonadectomy.

The patient receives hormone replacement therapy of testosterone at a dosage of 200 mg intramuscularly, and currently awaits masculinizing genitoplasty. He continues to receive follow-up with a multi-professional approach, which provides him with a better quality of life.

DISCUSSION

In this study, the patient was diagnosed with ovotesticular disorder of sex development (OT-DSD). This case was defined late after the diagnostic investigation of the disease, based on the clinical and laboratory findings of the patient. Early diagnosis is of

The karyotype examination is considered a primary tool in understanding the chromosomal constitution of patients with ovotesticular disorder of sex development. In OT-DSD individuals that are diagnosed early with karyotype 46, XX, the female sex is suggested as the best social sex option, due to the possibility of preserving the functioning ovarian tissue[8,9]. OT-DSD individuals with karyotype 46, XY or chimerism should consider the option of the male social sex, especially when there is an absence of a uterus and vagina, good phallic development and presence of testis on one side and a contralateral ovary[8,10].

The result of the chromosomal analysis of the patient in this study was 46, XX for all cells analyzed. Contrary to what happens in most cases of OT-DSD with karyotype 46, XX, the reported patient chose to maintain the social sex of his birth certificate, i.e., that of the male sex. This can probably be explained by the fact that diagnostic confirmation was not performed at an early age and despite having female sexual structures, correction surgery was chosen to adapt the appearance and functionality of the genitalia to the male sex, since the social sex of registration/upbringing was of paramount importance in his choice.

Despite the importance of karyotype examination in OT-DSD, the molecular investigation of the SRY gene is also relevant in the diagnosis of these patients. The SRY gene is located in the short arm of the Y chromosome and is considered fundamental in the coding of testicular determination factor (TDF), in addition to activating the differentiation of Sertoli cells, and is also a precursor of a gene activation cascade, which interacts with other genes to promote testicular differentiation[11].

In the present study, the OT-DSD 46, XX patient presented as SRY-negative. Recent studies indicate that the SRY gene is permissive, but not mandatory for testicular differentiation[5,7,12]. OT-DSD 46, XX Individuals that are SRY-negative can be explained by several mechanisms: increased expression of pro-testicular genes, such as SOX3, SOX9 and SOX10 genes; insufficient expression of pro-ovarian genes, such as RSPO1 and WNT4, and mutation in the NR5A1 gene, which is responsible for regulating gonadal and adrenal development[7, 13-16]. It is possible to consider that such mechanisms can overcome the absence of SRY and, consequently, initiate the specific pathway of testicular sexual differentiation. However, molecular analyses to elucidate which genes are involved in the sexual determination pathway of this type of patient are necessary.

CONCLUSION

Herein, we reported a patient with OT-DSD presenting a 46,XX karyotype and SRY-negative, which is considered a rare condition among DSD. Cytogenetic and molecular analyses were fundamental to assist in diagnosis, prognosis and genetic counseling. However, molecular analyses are necessary to elucidate which genes are involved in the sexual determination pathway of this patient.

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