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Disorders of Sex development 46, XY SRY (-) *vs.* 46, XX SRY (+): About 2 Cases

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Abstract

Introduction: Disorders or anomalies of Sex differentiation or Development (DSD),are congenital conditions during which the chromosomal, gonadal, and anatomical sexes are atypical. Cytogenetics and molecular genetics are essential in the classification of these variations by Karyotyping and searching for variants. Thus, we selected 2 patients of female civil status among those sent for diagnosis of a DSD and aim to do karyotype but also to verify the presence of the steroid genic region of the Y chromosome (SRY) by Polymerase Chain Reaction (PCR).

Materials and methods: We proceeded to a peripheral venous blood sample in a heparinized tube for the karyotype, and another tube with Ethylene Diamine Tetra Acetic acid (EDTA) for DNA extraction to search for the presence of the SRY gene by PCR.

Results: At the karyotype, the first patient had a formula 46, XY and the second a formula 46, XX. After PCR amplification of the SRY gene and electrophoretic migration on agarose gel, patient the first patient was SRY (+) and the second SRY (-).

Conclusion: Cytogenetics has a fundamental place in the classification of disorders of sexual development. Our patients could be classified as 46, XY DSD, as 46, XX DSD. The search of the SRY gene by PCR constitutes a good starting point in the molecular search for the etiological diagnosis of these abnormalities or variations in sexual differentiation.

Keywords: Sexual differentiation • Karyotype • Molecular genetics • Gonadal dysgenesis

Introduction

Sexual differentiation is induced by a series of molecular events leading to the transformation of the undifferentiated gonad into a testis or ovaries. This differentiation is led by a cascade of events depending on genetic and hormonal factors [1-4]. Indeed, the genetic sex is determined at fertilization, according to the sex chromosome provided by the sperm. Gonadal sex establishment is under genetic control due to the presence of the SRY gene. Following the action of the SRY gene (Y chromosome), a cascade of other genes is activated in the testis. In the absence of SRY and the presence of two X chromosomes, the gonad differentiates into the ovary [5,6]. Disorders of sexual differentiation are congenital conditions in which the chromosomal, gonadal, and anatomical sexes are atypical. Their etiologies are multiple and somewhat unknown in our context. Cytogenetic is essential in the diagnosis of these variations by Karyotype classification. Beyond conventional cytogenetic that allows to situate patients according to the Chicago 2005 classification, molecular genetics makes it possible to refine the etiological diagnosis of these variations by searching for mutations in sexual differentiation genes. In this work, we present the results of two (2) cases that we received as part of managing a disorder of sexual differentiation and whose karyotype was discordant with the search for SRY.

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Case Series

We selected two (2) patients of female civil status sent to the Histology-Embryology-Cytogenetics department of the Aristide Le Dantec hospital in Dakar for karyotype as part of the management of a disorder of sexual differentiation. After obtaining their free and informed consent, we carried out an interview then a clinical examination. We took a sample of peripheral venous blood on a heparinized tube for the karyotype and an EDTA (Ethylene Diamine Tetraacetic) tube for the molecular genetics. We carried out a conventional karyotype technique in 0.5 ml venous blood sample. The analysis of mitoses was carried out using Cytovision CW4000* Karyotyping software. To search for the SRY gene, Deoxyribonucleic Acid (DNA) extraction was carried out using the Zymo-search* kit and amplification was carried out with a PCR mix (25 microL): 1microL of primer (sense and antisense) + 12.5 MicroL of Mastermix*, 9.5 microL of MilliQ water + 2 microL of DNA. The primer sequence used and PCR conditions are available on request. The PCR mix was migrated on agarose gel before visualized using smartDoc* software.

Results

Patient N°1 was aged 23 and declared female in the civil registry. The karyotype indication is “sexual ambiguity on male secondary sexual characteristics”. On physical examination, we noted a general android morphotype with an absence of development of the mammary gland (Tanner grade 1) in the thoracic level (Figure 1) and the presence of a developed

genital tubercle (Prader grade IV), an empty scrotum, and developed pubic hair at the level on the external genitalia. We also note the presence of a single urogenital orifice (Figure 2). The result of the search for gonads by ultrasound was "absence of gonads after scanning the abdominal, pelvic and inguinal regions with a deep or superficial probe." The karyotype result was 46, XY, and SRY search was negative.

Patient N° 2 is aged 20 and declared female in the civil registry. The karyotype's indication was "sexual ambiguity evolving since birth associated with primary amenorrhea, presence of testicles and peniform clitoris." The general morphotype is gynoid. On examination of the thoracic level, Tanner stage 5 breast development is noted (Figure 3); at the level of the external genitalia, pubic hair is developed (Tanner stage 4), clitoral hypertrophy (Prader stage 1), two (2) palpated masses (one at the pubic level on the right, one at the inguinal level on the left and the presence of 2 urogenital holes (Figure 4). On ultrasound: "absence of internal female genital organs." Karyotype is 46, XX and SRY test came back positive.

Figure 1. Patient N°1 with a male morphotype and a single urogenital orifice.

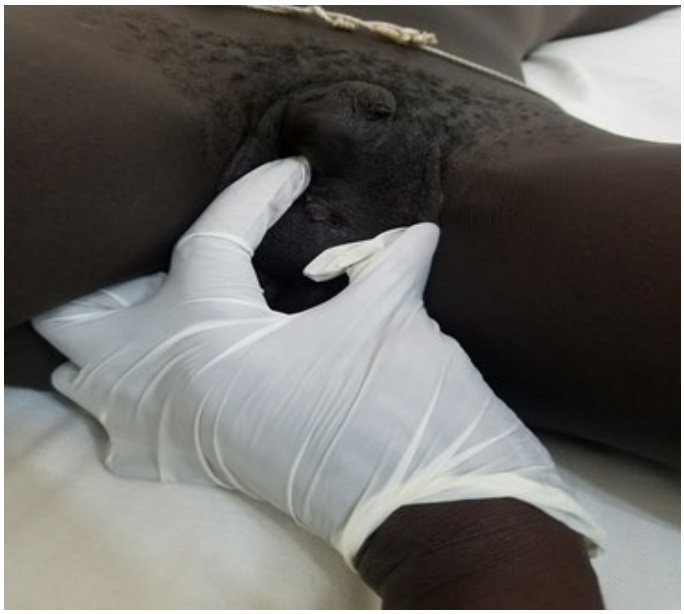

Figure 2. Patient N°1 with a male morphotype and a single urogenital orifice.

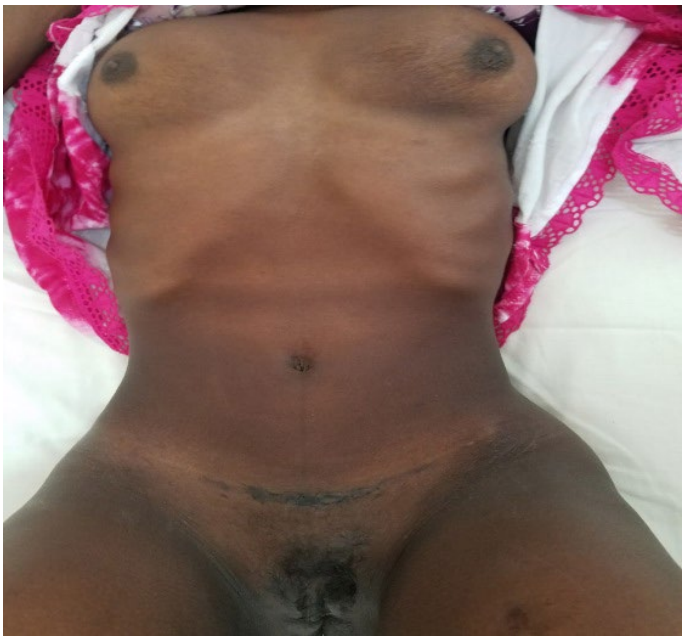

Figure 3. Patient N°2 with a female morphotype.

Figure 4. Patient N°2 with a female morphotype.

Discussion

The Chicago classification distinguishes anomalies of sexual differentiation into three categories depending on the Karyotype results: 1) chromosomal ADS including a sexual anomaly associated with an abnormality of the chromosomal formula such as Turner syndrome (45, XO), Klinefelter (47, XXY); 2) 46, XY ADS involving a sexual anomaly in a subject with chromosomal formula 46, XY which may result either from an anomaly of synthesis or action of androgens or from an anomaly of testicular development; 3) ADS, 46, XX implying a sexual anomaly with a chromosomal formula 46, XX referring either to an anomaly of ovarian development or to the action of an excess of fetal androgens [7,8].

Our patient N°1 was classified into the ADS 46, XY group, and the search for the SRY gene by PCR was negative. These results allow us to discuss the causes of testicular development abnormalities that may result from

complete or partial gonadal dysgenesis, testicular regression or ovotesticular DSD. 46, XY DSD with Complete Gonadal Dysgenesis (CGD) have an absence of testicular development. The testicle is replaced by a fibrous strip or a dysgenetic gonad, producing neither Anti-Mullerian Hormone (AMH) nor testosterone. Clinically, patients have female external genitalia without abnormalities and female genital tracts that are present (uterus and tubes). Patients come for consultation because they present primary amenorrhea. The karyotype carried out during the assessment shows a formula 46, XY. In the case of DSD 46, XY with Partial Gonadal Dysgenesis (PGD), the diagnosis is made in the neonatal period in children presenting an ambiguous phenotype, which can be very variable from one child to another depending on the quantity of testosterone produced by the testicle at the time of sexual differentiation. On clinical examination, we can find more or less severe hypospadias, micropenis, or scrotal abnormalities. Concerning the genital tract, patients may or may not have persistence of Müllerian ducts linked to insufficient secretion of AMH by Sertoli cells [9].

Furthermore, the absence of visualization of the SRY gene after PCR raises the question of the causes of partial gonadal dysgenesis at 46, XY, which can be induced by point mutations on the SRY gene or even mutations on NR5A1. Indeed, all the genes involved in the development of the bi-potential gonad or the development of the testis can be applied in cases of 46, XY DSD with CGD or PGD. However, the genetic cause is only found in about half of patients. The gene most frequently involved in gonadal dysgenesis 46, XY, is the SRY gene. Loss-of-function mutations are found in 10 to 15% of CGD cases [9-11].

The NR5A1 gene is responsible for 10 to 15% of cases of PGD [12] and rare cases of CGD. The NR5A1 gene has a nuclear receptor encoding an SF1 (Steroidogenic factor 1) protein. This protein is a nuclear receptor of a particular type, regulating the transcription of many enzyme genes involved in the steroidogenesis of adrenal and gonadal tissues [13]. SF1 also regulates pituitary protein hormone genes (beta subunit of LH (Lutein Hormone) and FSH (Folliculo-Stimulating Hormone) or testicular (AMH in particular).

Other genes involved in 46, XY DSD may be responsible for syndromic or non-syndromic DSD (Table 1) [9]. Furthermore, 46, XY DSD with PGD or CGD have been described in patients with a rearrangement in the regulatory regions of a gene [14]. Patient N° 2 allows us to discuss the causes of DSD 46, XX with testicular development. 46, XX DSD with testicular development is about 1/20,000 [9] and according to the phenotype, patients can be classified into three (3) categories:

1- Patients with male morphotype without abnormalities of the external genitalia or genital tract.

2- XX patients with ambiguity of the external genitalia

3- Patients with ovotesticular product [15].

Molecular abnormalities found in those three categories are very different. Patients with male product without abnormalities are the most common and represent approximately 85% of cases of DSD 46, XX with testicular development. The diagnosis is often made in adulthood because patients present infertility due to azoospermia. In 80 to 90% of cases, patients carry the SRY gene at the end of the short arm of the X chromosome due to unequal recombination during paternal meiosis [15-17].

In 15% of cases of testicular or ovotesticular DSD 46, XX, the diagnosis is made in the neonatal period due to ambiguous of external genitalia. In these cases, the SRY locus is only present in 15% of patients. The SRY test of our patient N°2 came back positive. The presence of the SRY locus in 46, XX patients with testicular development has long been the only identifiable genetic cause, but other causes of 46, XX DSD with testicular development are identified [9]. The RSPO1 gene has been implicated in a syndrome with palmar-plantar dyskeratosis, and the occurrence of squamous cell cancer associated with testicular development in 46, XX subjects [18]. The involvement of the WNT4 gene in human ovarian development has been demonstrated through the study of patients presenting a duplication of WNT4 associated with sexual reversion in XY subjects. The WNT pathway is a positive determinant of ovarian

Table 1. Genes involved in DSD 46, XY with CGD or PGD.

Gene	Locus	Transmission Mode	Phenotype	Frequence
SRY	YP11.3	Y	CGD (ou PGD)	10-15%
MAP3K1	5q11.2	Autosomal Dominant (AD)	CGD (ou PGD)	13-18%
NR5A1	9q33	AD	PGD (ou CGD)	10-15%
NR0B1	Xp21.3	RLX	CGD	Rare
DMRT1	9p24.3	AD (délétion)	CGD (ou PGD)	Rare
CBX2	17q25.3	Autosomal Recessive (AR)	Ovarian development	Rare
DHH	12q13.1	AR/AD	CGD (ou PGD)	Rare
WT1	11p13	AD	Frasier Syndrome, Deys-Drash Syndrome,	Rare
			WAGR Syndrome	
GATA4	8p23.1	AD	Heart defects, testicular abnormalities	Rare
SOX9	17q24.3	AD	Campomelic dysplasia,	Rare
			CGD ou PGD	
ATRX	Xp21.1	RLX	Alpha-thalassemie, Linked X mental retardation, PGD ou CGD	Rare
ARX	Xp21.3	RLX	Lissencephaly, CGD ou PGD	Rare
DHCR7	11q13.4	AR	Smith-Lemll-opitz syndrome	Rare
WNT4	1p36.1Y2	Duplication	PGD ou CGD syndromique	Rare

Table 2. Genes and regulatory regions involved in 46, XX DSD with testicular or ovotesticular development.

	Gene or region	Locus	Transmission Mode	Phenotype	Frequence
Gene	SRY	Yp11.3	Unbalanced Y translocation	Testicular development	80%
	SOX9	17q24.3	Duplication	Testicular development	Rare
	RSPO1	1p34.3	AR	Palmar-plantar hyperkeratosis, squamous cell carcinoma, testicular development	Rare
	SOX10	22q13.1	Duplication	Testicular or ovotesticular development	Rare
	SOX3	Xq27.1	Duplication	Testicular development	Rare
	WNT4	1p36.12	AR AD	SERKAL syndrome, Müllerian aplasia, hyperandrogenism	Rare
Regulatory regions	Upstream SOX3	Xq27.1	Deletion	Testicular development	Rare
	Upstream SOX9	17q24.3	Duplication	Testicular development	10%

determinism [19]. Homozygous WNT4 mutations in 46, XX patients can lead to SERKAL syndrome, while heterozygous mutations are responsible for a syndrome associating Müllerian agenesis with hyper secretion of testosterone but without testicular development; patients have a female phenotype. Until the end of the 2000s, only the SRY gene was identified in cases of DSD 46, XX with testicular development without abnormality of the external genitalia (Table 2) [9]. In 2011, several publications reported cases of DSD 46, XX with testicular development had found duplication in a gene desert upstream of the SOX9 gene [20,21]. All repeats identified in these patients had a common minimum region of 78 kb and were named RevSex. This region is located approximately 550 kb upstream of SOX9.

Conclusion

Based on the Chicago classification using the karyotype, cytogenetic has become essential for the search for variation of sexual differentiation causes but is not sufficient in some instances. Indeed, several genes involved in sexual differentiation and genotypic variations resulting from their sequences could induce phenotypic variations. Thus, for complete etiological research, cytogenetic will have to use molecular genetics and vice versa, as evidenced by the cases that we report in this work where the karyotype results were discordant with the search for SRY.

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All authors have agreed to the publication of this report.

Conflict of Interest

No financial interest or any conflict of interest exists.

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