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FISH, PCR and cytogenetic characterization in a girl with ambiguous genitalia and karyotype mos46,X,iso(Y)(qter→p11.3::p11.3→qter)[80]/45,X[17]/46,X,+mar[3]

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ABSTRACT. A cytogenetic study was carried out in a girl with virilized external genitalia, who showed a karyotype containing a Y isochromosome in mosaic form: mos46,X,iso(Y)(qter→p11.3::p11.3→qter)[80]/45,X[17]/46,X,+mar[3]. The chromosome aberrations were confirmed by fluorescence *in situ* hybridization analysis, with both whole chromosome paint Y probe and centromeric X chromosome probe. The molecular analyses by PCR detected the presence of the SRY, DAZ and AMGY genes, confirming the presence of the whole long arm and almost whole short arm of the Y chromosome. We suggest that the structural alteration of the Y chromosome was a new mutation, which occurred in the initial mitotic divisions of the embryo, originally 46,XY. The breakpoints occurred on the distal extremity of the short arm with later fusion of its extremities producing a Y isochromosome. The later numerical alteration occurred as a consequence of chromosomal instability. Although almost all cells (80%)

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in peripheral blood belonged to the iso(Y) line with a duplicated SRY gene, this did not determine male sexual differentiation in the patient. The result of accurate evaluation provides correct sex assignment and the prevention of the neoplastic degeneration of a dysgenetic gonad.

Key words: Ambiguous genitalia; Y isochromosome; Mosaicism; SRY gene

INTRODUCTION

Disorders of sexual differentiation have been associated with numerical and structural aberrations on chromosomes X and Y such as Turner's syndrome and mixed gonadal dysgenesis. Monosomy of X represents 50-60% of the karyotypes observed in gonadal dysgenesis (Álvarez-Nava et al., 2003). Isochromosomes are the most commonly reported aberrations of the human Y chromosome. As they are unstable during cell division and can generate various types of cell lines, most patients reported are chromosomal mosaics, generally including a 45,X cell line. Phenotypic variability depends on the location of the breakpoints as well as on the degree of mosaicism in the various tissues varying from male to abnormal female or individual with ambiguous genitalia (DesGroseilliers et al., 2006).

When the abnormality involves a rearrangement in the structure of the Y chromosome, the resulting phenotype will depend on i) the complexity of the rearrangement and the presence of the gene SRY, or the genes responsible for low stature and spermatogenesis, and ii) the proportion of the cell line 45,X in the different tissues. An association has been suggested between Y chromosome microdeletions and severity of the phenotype in 45,X/46,XY (Álvarez-Nava et al., 2008).

Mixed gonadal dysgenesis is a heterogenic group with chromosomal, gonadal, or anatomical sex being atypical. It is generally characterized by the presence of a dysgenetic testis on one side and counter-lateral vestigial gonad, with derived müllerians and different degrees of genital ambiguity. In most cases, the karyotype is a mosaic 45,X/46,XY, with a Y chromosome normal or anomalous (Canto et al., 2004).

The presence of Y chromosome sequences means that patients with dysgenetic gonads, including Turner's syndrome and mixed gonadal dysgenesis, have an increased risk of developing germ cell tumors such as gonadoblastoma (Robinson et al., 1999). Frequently in gonads of patients with disorders of sex development the germ cells show delayed maturation. This phenomenon may be related to the risk of malignant transformation. The presence of a well-defined part of the Y chromosome (known as the GBY region) is a prerequisite for malignant transformation (Looijenga et al., 2007).

Herein, we describe a child with ambiguous genitalia with a female phenotype who was investigated by high-resolution karyotyping, fluorescence *in situ* hybridization (FISH) and polymerase chain reaction.

SUBJECTS AND METHODS

This study was approved by the Ethics Committee of the University Hospital/UFMA (CONEP 7940), and an informed consent form was signed by the child's guardian. The patient

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had a cesarean birth, weighing 3450 g and measuring 47 cm. The child presented ambiguous genitalia with hypertrophy of the clitoris since birth. Her parents were not consanguineous and there was no family history of such condition.

When the patient was four years old she was healthy in general, and she had a height of 103 cm and weighed 17.8 kg. The genitals exhibited clitoromegaly (2.5 cm), fusion of the posterior labia, and non-palpable gonads, and the orifice of the urethra was not visible. At 7 years old, she showed a bone structure compatible with chronological age, and the supra-renal glandular function was normal. Pelvic ultrasound revealed a normal uterus, compatible with age and no ovaries were visible.

Cytogenetic analysis

A short-term culture of peripheral blood lymphocytes was carried out for conventional (Ford and Hamerton, 1956) and for high chromosomal resolution analysis (Yunis, 1976). One hundred metaphases were analyzed with GTG banding for the determination of the family's karyotypes (proband, her mother and her father). The karyotypes of the parents were found to be normal. The patient presented a mosaic karyotype with three cell lineages: mos46,X,iso(Y)(qter→p11.3::p11.3→qter)[80]/45,X[17]/46,X,+mar[3] (Figure 1).

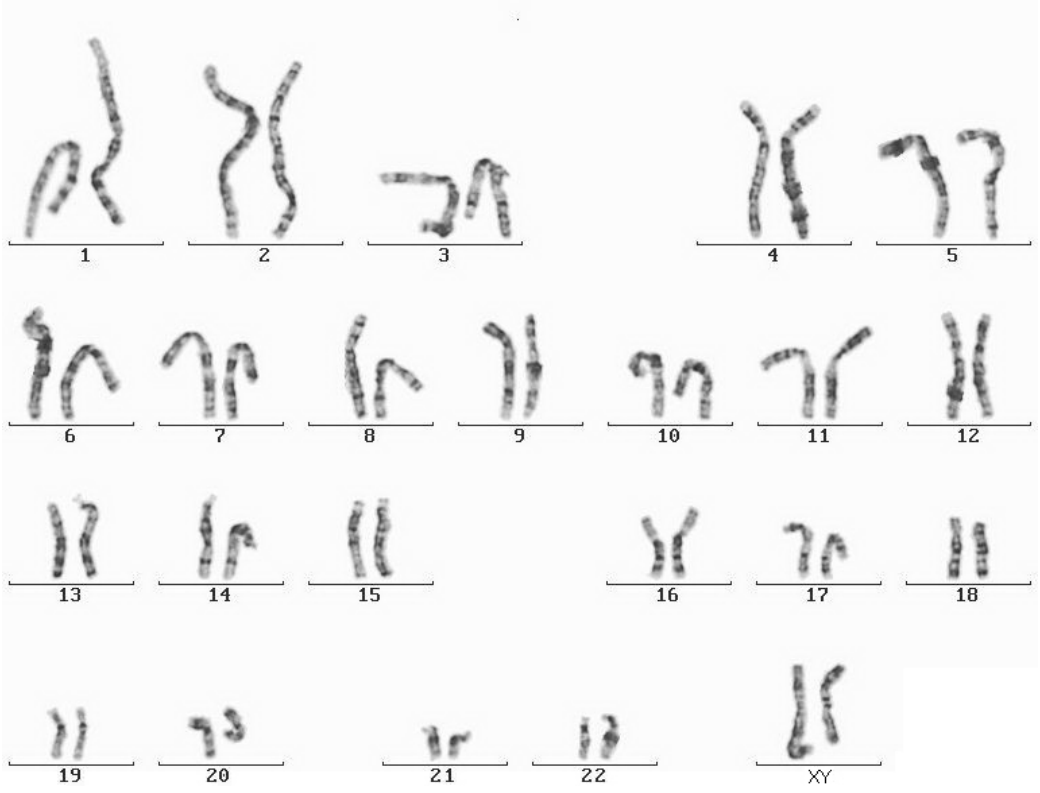

Figure 1. Karyotype of patient presenting Y isochromosome.

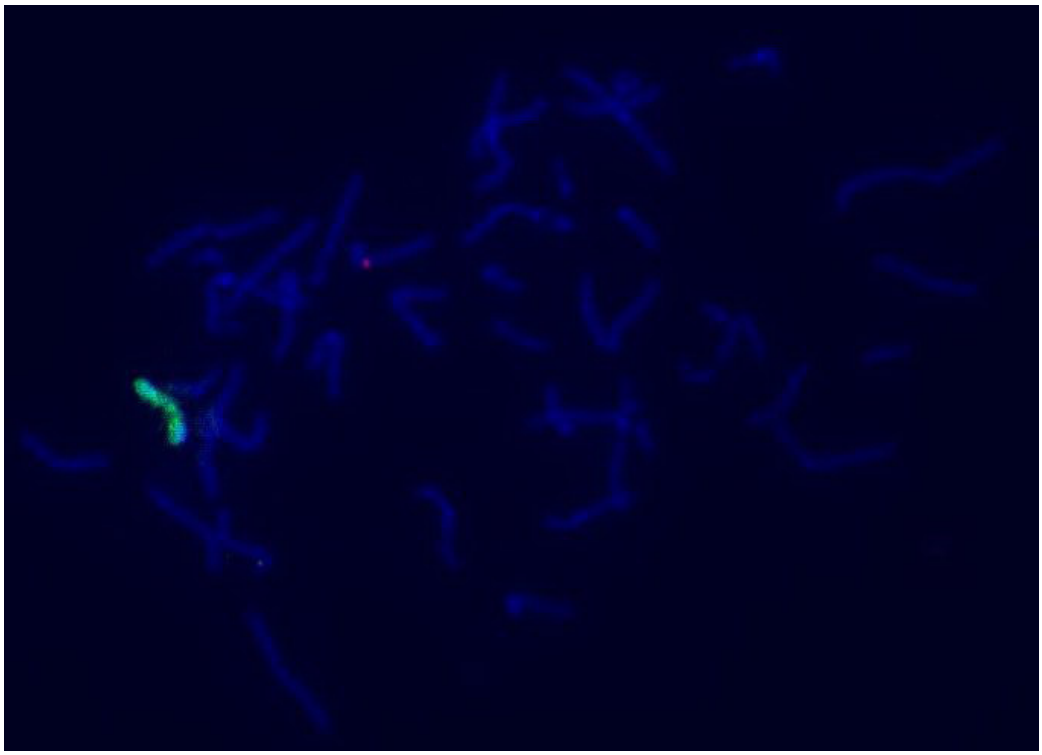

Figure 2. Metaphase showing Y isochromosome (green) and centromere of X chromosome (red).

Fluorescent *in situ* hybridization

FISH was performed on lymphocytes from peripheral blood, following the protocols of the supplier (Vysis Incorporation, USA). A red centromeric probe was used for the X chromosome and a green paint probe for the Y chromosome. The analysis confirmed the presence of a cell lineage containing material from Y chromosome and another lineage carrying X chromosome monosomy (Figure 2).

Polymerase chain reaction

DNA was extracted from peripheral blood (Sambrook et al., 1989). The detection of sequences from Y chromosome was performed using specific primers for the SRY gene (Innis et al., 1990), localized on Yp11.3 (Sinclair et al., 1990; Vilain et al., 1992), AMGY on Yp11.2 (Vollrath et al., 1992) and DAZ on Yq11.23 (Reijo et al., 1995; Saxena et al., 1996). The β-globin gene localized on chromosome 11 (Guerreiro et al., 1992) was used as control. The amplified products were separated by agarose gel (2%) electrophoresis. The results revealed the presence of all genes investigated on the Y chromosome (Figure 3).

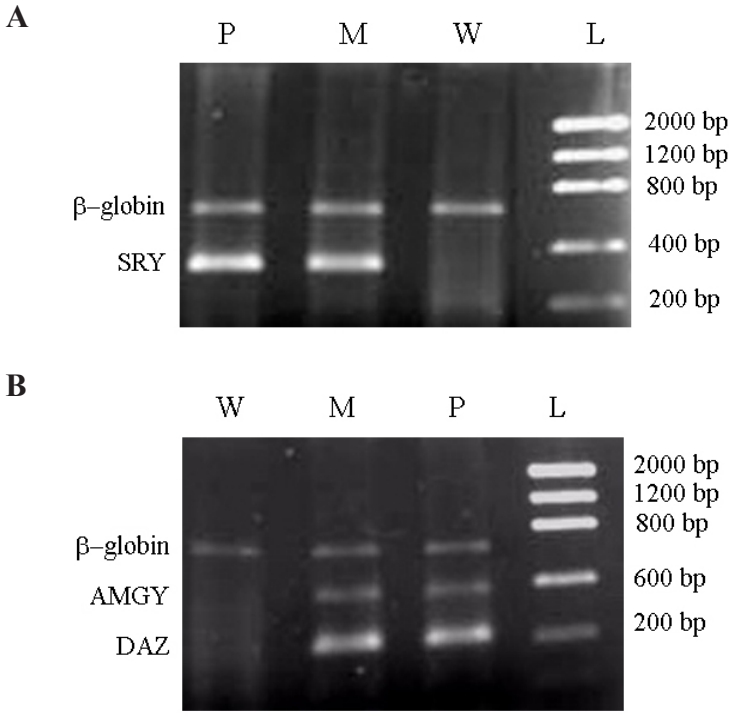

Figure 3. A. Amplification of SRY. **B.** Amplification of DAZ and AMGY. P = patient; M = man; W = woman; L = ladder.

DISCUSSION

We describe here a patient who presented virilization of external genitalia since her birth but no signals of Turner's syndrome. At 7 years old, the child showed a female phenotype and a development compatible with her age. She has a uterus, but her gonads are not visible. She has a mosaic karyotype in peripheral blood lymphocytes with three lineages as described: mos46,X,iso(Y)(qter→p11.3::p11.3→qter)[80]/45,X[17]/46,X,+mar[3]. FISH analyses confirmed the presence of two lineages, one of them with Y isochromosome and the other one with just one X chromosome.

The breakpoints determined by high chromosome resolution technique were similar to those observed by other authors (Marcus-Soekarman et al., 2005), although in this cited paper an abnormal external genital in a male fetus was described. It was suggested that the resulting phenotype of mosaicism depends on the exact region of the breakpoint as well as the proportion of each cell lineage in the different tissues. If this is found close to the SRY gene, in Yp11.2 or p11.3, the phenotype will be female. If the cell line 45,X is not predominant and the breakpoint is on the long arm, the individual could have two intact copies of the SRY gene and will generally be male (DesGroseilliers et al., 2006).

The phenotypic spectrum (clinical and gonadal features) from patients with a 45,X/46,X,mar(Y) karyotype depends in part on the prevalence, time occurrence, and distribution of the 45,X cell line. Nine patients presenting isodicentric Y chromosome were recently reported, and two of them were males and had a non-mosaic karyotype, while the third male was a mosaic with a predominant 46,XY cell line. Three of the females had a major 45,X cell line, while the other two females and the patient with ambiguous genitalia had a major 46,X,idic(Y) cell line. Analyses of gonadal tissues from these last patients gave results concordant with their phenotypes (DesGroseilliers et al., 2006).

On the other hand, Guedes et al. (2006) reported on a girl who, despite her 45,X/46,X,idic(Yp) karyotype, showed no signs of virilization or physical signs of Ullrich-Turner syndrome, except for a reduced growth rate. They observed a pronounced difference in the distribution of the mosaicism between the two tissues analyzed. Although almost all cells (97.5%) in peripheral blood belonged to the idic(Yp) line with a duplicated SRY gene, this did not determine any degree of male sexual differentiation in the patient, as in the gonads the predominant cell line was 45,X (60%).

Another paper reported a fetus with a mosaic karyotype, 45,X/46,X,idic(Y)(qter-p11.3::p11.3-qter), with unambiguous male external genitalia and a defect in the interventricular septum of the heart. A markedly higher percentage of Y-containing cells was observed in the gonads (55%) than in the amniotic fluid (17%) and placental villi (11%), which was considered to be the major reason why the fetus did not have ambiguous genitalia (Wu et al., 2007). Thus, the phenotypes displayed by these patients vary from female, without signs of virilization, to female with varying degrees of virilization, depending on the presence or absence of the SRY gene and, maybe more importantly, the degree of mosaicism and the tissue distribution of the 45,X lineage (Fernandez and Pasaro, 2006).

In the present case, the presence of three lineages, including 45,X (17%), another one (3%) showing a marker chromosome, and the majority of cells (80%) having a Y isochromosome, led us to suggest that a structural alteration of the Y chromosome was a new mutation, which occurred in the initial mitotic divisions of the embryo, originally 46,XY. The breakpoints occurred on the distal extremity of the short arm with later fusion of its extremities producing an isochromosome. In this way, this chromosomal aberration contains the SRY gene located in Yp11.3. The small marker being unstable was found in minor frequency. The later numerical alteration occurred as a consequence of chromosomal instability. Molecular analyses detected the presence of the SRY, DAZ and AMGY genes, confirming the presence of the whole long arm and almost all the short arm of the Y chromosome with duplicated SRY gene.

A newborn with mixed gonadal dysgenesis with persistence of müllerian ducts, two right testicles, one vestigial gonad on the left and the absence of Wolffian derivatives has been reported (Queipo et al., 2005). The karyotype 45,X/46,X,idic(Yp) indicates that the breakpoint occurred in Yq, preserving the SRY gene. The phenotype shown by the patient in this case is female, without apparent stigmata of Turner's syndrome.

The diagnosis of Turner's syndrome, in about 10% of cases, only happens in adulthood (Elsheikh et al., 2002). Nevertheless, women with dysgenetic gonads and having material belonging to the Y chromosome have a risk of developing gonadoblastoma, which is higher by a factor of 15-20%, and so they should be submitted to surgery to remove the gonads (DesGroseilliers et al., 2006). Special investigations are recommended for newborn with perineoscrotal hypospadia and bilateral or unilateral testicular maldescent immediately after birth.

The result of accurate evaluation provides correct sex assignment and the prevention of the neoplastic degeneration of a dysgenetic gonad (Ságodi et al., 2007).

Although many of the pathways regulating sexual differentiation have been elucidated, direct downstream targets of SRY are still unclear, making a top down approach difficult. However, recent study has demonstrated that the fate of the gonad is actively contested by both male-promoting and female-promoting signals. Sox9 and Fgf9 push gonads towards testis differentiation. These two genes are opposed by Wnt4, and possibly RSPO1, which push gonads toward ovary differentiation (Dinapoli and Capel, 2008).

Studies of mosaics involving sex chromosomes, especially when there is involvement of the SRY gene, may help us to understand the complex process of mammalian sex determination. Herein we describe a female patient presenting external virilized genitalia with the greater part of her cells having a Y isochromosome, with the persistence of the SRY gene. Even though she carries the SRY gene, the mosaic characteristics of her karyotype make this insufficient to induce the masculine phenotype. We agree with other authors who affirm that phenotypes vary from female, without signs of virilization, to female with varying degrees of virilization, depending on the presence or absence of the SRY gene and, maybe more importantly, the degree of mosaicism and the tissue distribution of the 45,X lineage.

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