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Molecular analysis of an idic(Y)(qter→p11.32::p11.32→qter) chromosome from a female patient with a complex karyotype

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ABSTRACT. A female patient with a structurally abnormal idic(Y)(p11.32) chromosome was studied using fluorescence *in situ* hybridization and PCR to define the precise position of the breakpoint. The patient had a complex mosaic karyotype with eight cell lines and at least two morphologically distinct derivatives from the Y chromosome. The rearrangement was a result of a meiosis I exchange between sister chromatids at the pseudoautosomal region, followed by centromere misdivision at meiosis II. Due to instability of the dicentric Y chromosome, new cell lines later arose because of mitotic errors occurring during embryonic development. Physical examination revealed a normal female phenotype without genital ambiguity, a normal uterus and rudimentary gonads which were surgically removed.

Key words: 45X, Mosaicism, Dicentric Y, Sex determination, Turner syndrome

INTRODUCTION

Turner syndrome is a genetic disorder caused by the haploinsufficiency of certain normally expressed genes from both X chromosomes, the active and the inactive, and from the Y chromosome. The Turner phenotype includes short stature, ovarian failure, pterygium colli, cubitus valgus, lymphedema, aortic coarctation, and renal anomalies, among others (De la Chapelle, 1990).

When the karyotype is determined by molecular techniques, approximately 25-30% of Turner patients have a 45,X karyotype (Fernandez et al., 1996) and the remainder are demonstrable mosaics for a cell line containing a second sex chromosome. This is usually an X chromosome, but in about 6-12% of the cases, a second cell line containing a Y chromosome is present (Verp and Simpson, 1987). The presence of the pericentromeric Y sequences in such patients with gonadal dysgenesis is clinically crucial, since they have an increased risk of developing gonadoblastoma (Page, 1987).

On the other hand, the most common abnormal Y chromosome is a dicentric fragment present as part of a mosaic karyotype including a 45,X cell line (Robinson et al., 1999). They are usually generated during gametogenesis before spermatid formation or during the first division after fertilization, and most are present as part of a mosaic karyotype. The resulting phenotype ranges from female to male, depending on the presence or absence of the testis-determining gene *SRY* and, perhaps more importantly, on the degree of mosaicism and the tissue distribution of the 45,X cell line.

The present study reports the results of an extensive molecular and cytogenetic study of the Y chromosome from a 4-year-old female with a mosaic karyotype: 45,X/46,X,del(Y)(p11.32)/46,X,idic(Y)(qter→p11.32::p11.32→qter)/47,XX,del(Y)(p11.32)/47,X,2del(Y)(p11.32)/47,X,del(Y)(p11.32),idic(Y)(qter→p11.32::p11.32→qter)/47,XX,idic(Y)(qter→p11.32::p11.32→qter)/47,X,2idic(Y)(qter→p11.32::p11.32→qter).

SUBJECT AND METHODS

Patient

The patient was a 4-year-old female with a complex mosaic karyotype showing eight cell lines and at least two morphologically distinct derivatives from the Y chromosome. She was diagnosed accidentally at the age of 4 years. Physical examination revealed a normal female phenotype without genital ambiguity, a normal uterus and rudimentary gonads which were surgically removed. At the age of 4.6 years, she measures 100.7 cm and her development and health are good. Intellectually, she has developed normally.

Cytogenetic and molecular analysis

Standard techniques for culturing lymphocytes from peripheral blood were used (Moorhead et al., 1960), and the preparations were treated with trypsin to obtain G-banding (Seabright, 1971).

FISH analysis

FISH analysis was performed on peripheral blood lymphocytes according to the stan-

dard procedure, using the probes: DYZ1 (Oncor) which specifically hybridizes to the Yq12 region-satellite DNA; DYZ3 (Oncor), a specific probe for the Y-centromeric region; DXZ1 (Oncor), specific for the X-centromeric region; DXZ4 (Oncor), specific for the repetitive DNA in Xq24; SHOX probe cosmid LLNOYCO3'M'34F5, kindly provided by Dr. Andrew Zinn, and a telomeric probe for hybridization to telomeres of all chromosomes (Meyne and Moyzis, 1994).

DNA analysis by PCR

DNA extraction from peripheral blood and ovarian tissues was carried out using standard procedures. Three sets of oligonucleotide primers were used in the PCR reaction: YC1-YC2 to amplify a 170-bp fragment from the centromeric region of the Y chromosome (Witt and Erickson, 1989); XES7-XES2 to amplify a fragment of 609 bp from the *SRY* open-reading frame (Berta et al., 1990), and DYZ1A-DYZ1B to amplify a 1024-bp fragment from the DYZ1 region contained within the Yq12 (Cooke, 1976).

For the molecular study of hidden mosaicism, several polymorphic STS were used [NCBI BANK (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists)] (Table 1). The results were obtained on polyacrylamide gels (GeneGel Excel 12.5/24 Kit from Amersham Pharmacia Biotech) run at 600 V for 30-40 min.

Table 1. Description of primers used in PCR analysis.

Name	Sequence	Annealing	Fragment	Localization
YC1-YC2	5'-ATGATAGAAACGGAAATATG-3' 5'-AGTAGAATGCAAAGGGCTCC-3'	57º	170 bp	centromere
XES7-XES2	5'-CCCGAATTCGACAATGCAATCATATGCTTCTGC-3' 5'-CTGTAGCGGTCCCGTTGCTGCGGTG-3'	65º	609 bp	Yp11.3
DYZ1	5'-AATTTGAGCATTCGTGTCCATTCT-3' 5'-AATGCCCTTGAATTAAATGGACT-3'	60º	1024 bp	Yq12
DXYS233	5'-TGGGAATTCGAGGCTG-3' 5'-TGATTTCCATCCTGGGGT-3'	65º	polymorphic	PAR1
DXYS14	5'-AGTTGCTCTCTCTTCCACAAACA-3' 5'-AGTCATGAGAATGTGCTGGAGCT-3'	54º	polymorphic	PAR1
SHOX-CA	5'-CATGTCATATATATATGTGATCC-3' 5'-CAGACAGAAATCCTTCATAAA-3'	50º	polymorphic	PAR1
DXYS15	5'-TATTTATGGAAATTGCCCCC-3' 5'-TAATACAAGCCAGACGAGCC-3'	56º	polymorphic	PAR1
DXYS234	5'-CCCAGATCGCNCCATT-3' 5'-ATGGCTCTGAGGCGGG-3'	50º	polymorphic	PAR1
DXYS228	5'-ATTAGCAGTTCACAGAGCCC-3' 5'-ACGTGGGAGCAATAGTTCA-3'	50º	polymorphic	PAR1
DXYS229	5'-TGTGGCTGTTGTAACAAATTACT-3' 5'-CCTAGGTTGCTGCAAATG-3'	53º	polymorphic	PAR1

RESULTS

A derived Y chromosome was found in a 4-year-old female with moderate short stature and gonadal dysgenesis. She exhibited a chromosome similar in size to a member of group D, which suggests two Y chromosomes united by the pter ends (Figures 1 and 2). The analysis of 206 metaphases by FISH revealed at least eight cell lines and two different derivatives from the Y chromosome. In 58% of the cells, a double-hybridization signal was observed in the derivative chromosome for probes DYZ1 and DYZ3, corresponding to double heterochromatic and centromeric regions, respectively (Figures 1A and 2). The cell line 45,X was found in 19% of the cells, whereas the cell line 46,X,del(Y)(p11.32) was present in 16.5%. Furthermore, five other cell lines were observed in smaller percentages, resulting from breakage of the idic(Y)(qter→p11.32::p11.32→qter) at p11.32 and later mitotic random distribution of the two del(Y)(p11.32) and the X chromosome:

- 3% corresponding to a cell line with an X chromosome, a Y chromosome with terminal deletion and an isodicentric Y: 47,X,del(Y)(p11.32),idic(Y)(qter→p11.32::p11.32→qter) (Figure 1B).
- 1.5% corresponding to a cell line containing two X chromosomes and one isodicentric Y chromosome 47,XX, idic(Y)(qter→p11.32::p11.32→qter).
- 1% of the cells showed a combination of one X chromosome and two isodicentric Y chromosomes 47,X, 2idic(Y)(qter→p11.32::p11.32→qter) (Figure 1C).
- 0.5% showed one X chromosome and two Y chromosomes with terminal deletion 47,X, 2del(Y)(p11.32) (Figures 1D and 2).
- 0.5% of the cells showed two X chromosomes and one deleted Y chromosome 47,XX,del(Y)(p11.32).

Molecular analysis showed that all the der(Y) had lost the PAR1 region, including the *SHOX* gene (in blood and ovarian tissues). The study of polymorphism using a panel of several PAR1 markers (Table 1) enabled the localization of the Yp breakpoint in the region between the *SHOX* and *SRY* genes. This same result was observed in lymphocytes and in ovary. PCR analysis also confirmed the presence of the Yq12 region, the Y-centromere and the *SRY* gene in blood and ovary.

Histopathological examination of the gonads showed bilateral atrophic fibrotic ovarian stroma with no signs of egg cells or testicular or tumor tissue.

DISCUSSION

In the present study, a combined PCR and FISH approach was taken to determine the break/joint point and the origin of an isodicentric Y chromosome found in a patient with rudimentary gonads and moderate short stature. The patient carried in 58% of the cells analyzed an isochromosome dicentric Y (qter→p11.32::p11.32→qter) characterized by the presence of two copies of the long arm, two copies of the centromeric region and two copies of practically the entire short arm (Figure 2). The structure had a break/joint point within Yp11.32, and had lost the pseudoautosomal region.

The patient also carried der(Y) chromosomes (one or two copies) with a deletion at

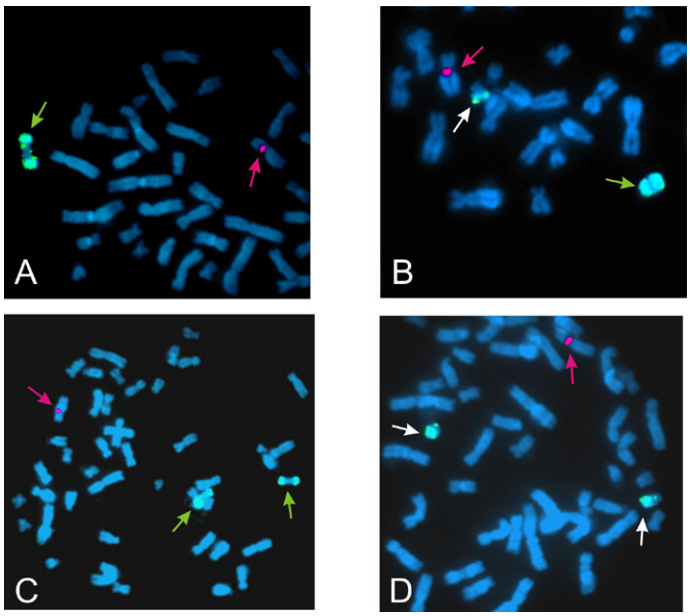

Figure 1. FISH analysis using DXZ1 (magenta signal) and DYZ1 (green signal). **A.** 46,X,idic(Y)(qter→p11.32::p11.32→qter) metaphase; the magenta arrow indicates the X chromosome and the green ones indicate the idic(Y)(qter→p11.32::p11.32→qter). **B.** 47,X,del(Y)(p11.32),idic(Y)(qter→p11.32::p11.32→qter) metaphase; the magenta arrow indicates the X chromosome, the white arrow indicates the del(Y)(p11.32) and the green ones indicate the idic(Y)(qter→p11.32::p11.32→qter). **C.** 47,X,2idic(Y)(qter→p11.32::p11.32→qter) metaphase; once again the magenta arrow indicates the X chromosome, and the green arrows indicate the two idic(Y)(qter→p11.32::p11.32→qter). **D.** 47,X,2del(Y)(p11.32) metaphase; the magenta arrow indicates the X chromosome, and the white arrows indicate the two del(Y)(p11.32).

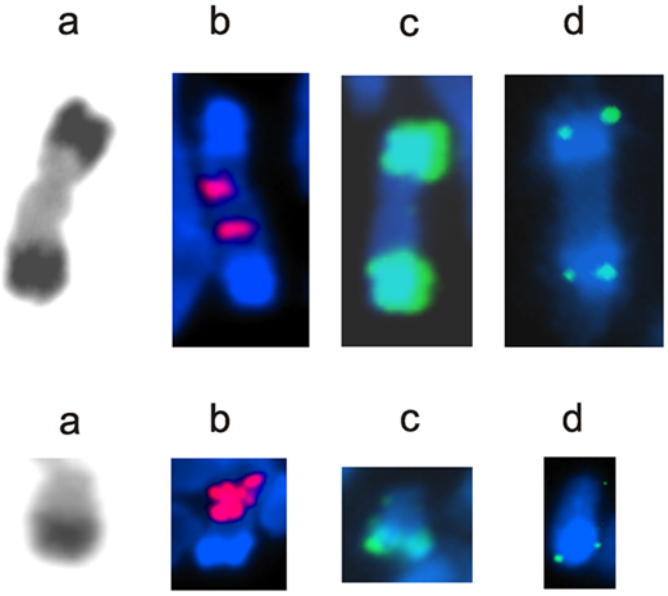

Figure 2. Characterization of the most common der(Y) found in this patient: idic(Y)(qter→p11.32::p11.32→qter) (upper) and del(Y)(p11.32) (lower). a) DAPI. b) Hybridization with DYZ3 probe. c) Hybridization with DYZ1 probe. d) Hybridization with the telomeric probe.

Yp11.32 (Figure 2) combined or not with the isodicentric Y chromosome (Figure 1), and one or two X chromosomes. The result was a complex karyotype formed by eight cell lines and at least two different der(Y).

All the structures derived from the Y chromosome are characterized as carrying the testis-determining gene *SRY*, the putative gonadoblastoma gene (GBY), thought to lie in the Y-pericentromeric region (Salo et al., 1995), and also a deletion in the PAR1 region. The fact that an intact 46,XY line was not found and that all the der(Y) had lost the PAR1 region suggests a meiotic origin for the dicentric Y. Perhaps the isodicentric Y chromosome was present in the sperm before fertilization as a result of an error during gametogenesis. Errors occurring after the first zygotic division would result in mosaicism including a normal cell line. We think that the dic(Y) was a result of a meiosis I exchange between sister chromatids at a side between *SRY* and the *SHOX*, followed by centromere misdivision in meiosis II. These data are in concordance with reports on isochromosomes by Battin (2003); Robinson et al. (1999), and Hsu (1994).

The patient showed a total of eight cell lines and at least two morphologically distinct abnormal Y derivatives, all presumably descendants of a progenitor and unstable idic(Y) chromosome. The heterogeneous cell content observed suggests a great mitotic instability of sex chromosome Y and mitotic non-disjunction.

Usually, isodicentric Y chromosomes occur in mosaic form and are generally considered unstable elements since improper alignment of two centromeres on the metaphase spindle may lead to the formation of a bridge during anaphase (Cohen et al., 1973). In the patient studied here, the isodicentric Y chromosome showed two noticeable centromeres. If both centromeres were active, it could be assumed that the Y derivatives observed in the different cell lines would be the result of the breakage of the isodicentric at Yp11:32 due to improper alignment of the two centromeres on the metaphase spindle.

As in all the cases studied in our laboratory and in most of the reports published to date (Robinson et al., 1999), the patient examined here had a 45,X cell line (19%). Such mosaic patients exhibit a phenotype ranging from female to male, depending on the presence or absence of the testis-determining gene *SRY* and, perhaps more importantly, on the degree of mosaicism and the tissue distribution of 45,X cells. It has been proposed that the predominance of XO or XY cells determines gonadal differentiation into a testis or a streak gonad (Bergada et al., 1986).

On the other hand, Turner syndrome is the result of haploinsufficiency of a specific gene(s) that must escape from X-inactivation, and second, these individuals must have a functional Y homolog. The discovery of the pseudoautosomal region at the termini of Xp and Yp fits well with these two requirements: meiotic recombination maintains nucleotide sequence identity between X- and Y-linked pseudoautosomal genes, and all such genes tested to date escape X-inactivation (Zinn and Ross, 1998). Nevertheless, the only Turner syndrome features present in this patient were short stature and gonadal dysgenesis. The absence of the PAR1 region in all cells examined in this patient suggests that loci responsible for other Turner features lie outside of the pseudoautosomal region (Joseph et al., 1996; Schwinger et al., 1996; Spranger et al., 1997; Haddad et al., 2003). Our data are in agreement with the fact that the only PAR1 gene consistently related to Turner syndrome is the short stature gene or *SHOX/PHOG*. This gene is a strong candidate for a Turner syndrome growth gene on the basis of its chromosomal location, its pattern of expression and mutational analysis (Rao et al., 1997; Alves et al., 2003).

In conclusion, it appears that the most common abnormal Y chromosome present in

Turner syndrome patients is an isodicentric Y chromosome occurring as part of a mosaic karyotype including a 45,X cell line. It is probable that isodicentric Y chromosomes are usually generated during gametogenesis before spermatid formation, or during the first division after fertilization, and that almost all are present as part of a mosaic karyotype. The Turner syndrome patients with a Y chromosome studied in our laboratory carried the testis-determining factor gene *SRY*, but the mosaic nature of their karyotypes rendered this insufficient to induce a male phenotype. In all our patients, the degree and distribution of the 45,X cell line seem to be decisive factors in phenotype determination.

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