Detailed analysis of an idic(Y)(q11.21) in a mosaic karyotype

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Abstract. Abnormalities involving sex chromosomes account for approximately 0.5% of live births. The phenotypes of individuals with mosaic cell lines that exhibit structural aberrations of the X and Y chromosomes are variable and difficult to predict. Phenotypes associated with sex chromosome mosaicism vary from females with Turner syndrome to males with infertility, and include individuals with ambiguous genitalia. In this study, we report a 17-year-old male with phenotypic features of Klinefelter syndrome with an isodicentric Y chromosome and a final karyotype of 45,X[4]/46,X,idic(Y)(q11.21)[95]/47,XX,+idic(Y)(q11.21)[1]. Application of high resolution molecular cytogenetic techniques as well as molecular studies revealed two copies of the sex-determining region of Y chromosome (SRY) gene and two centromers. Additionally, the breakpoint in Yq11.21 was narrowed down between positions 13.4 and 14.3 MB (hg18). We present a patient with partial disomy of Ypter to Yq11.21 in the majority of the patient cells, showing phenotypic features of Klinefelter syndrome. The syndrome may have occurred due to a more prominent presence of the cell line 47,XX,+idic(Y)(q11.21) detected only once in 1% of the peripheral blood cells. This finding may prove helpful in similar cases with symptoms of Klinefelter syndrome, but which exhibit an absence of the cell line 47,XXY in peripheral blood.

Introduction

Structural abnormalities with breakpoints in the euchromatic section of a Y chromosome may lead to its instability and loss in subsequent cell cycles, with formation of a mosaic 45,X cell line. As a result, numerous morphologic forms of the aberrant Y chromosome occur in the mosaic karyotype.

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Dicentrics are the most common structural abnormalities of the Y chromosome (1,2). They are mitotically unstable and may consequently be lost during development, resulting in the majority of patients with dicentric Y chromosome having a mosaic karyotype, mostly with a 45,X cell line (3). Clinical features of such patients may range from Turner-syndrome-like females to infertile males, depending on the structure of the dicentric Y chromosome, the sites of breakage and types of mosaicism.

The identification of Y chromosome-derived material in cases with dysgenetic gonads is associated with an increased risk of developing gonadoblastoma. The presence of a Y chromosome, or part of it, is associated with a 25-70% risk of gonadoblastoma, the highest risk being associated with mixed gonadal dysgenesis and increasing age (4). In patients with the 45,X/46,XY karyotype, the risk appears lowest in phenotypic males, intermediate (0.5%) in patients with ambiguous external genitalia and highest (22%) in phenotypic females (1).

The Y chromosome contains crucial loci for normal male sexual development. The sex-determining region of the Y chromosome (SRY), a transcription factor on the short arm of the Y chromosome, is the critical switch leading to testis development. Mutations in SRY result in XY individuals developing as females; patients with a 45,X karyotype with insertion of SRY into an autosome have a male phenotype (5,6). Genes in the euchromatic region of the long arm of the Y chromosome (Yq11) are required for normal spermatogenesis, and Y chromosome microdeletions are an important genetic cause of primary testiculopathies. Deletion mapping was directed at the identification of genes related to spermatogenesis, and defined three regions as the azoospermia factors (AZFa, AZFb and AZFc) mapped to Yq11 (7). The main candidate gene in AZFc is the DAZ (deleted in azoospermia) cluster, a set of genes transcribed in the testis and expressed exclusively in germ cells, apparently encoding an RNA binding protein (8).

In this study, we present a detailed molecular-cytogenetic characterization of a patient with phenotypic features of the Klinefelter syndrome with mosaicism involving an isodicentric Y chromosome.

Patient and methods

Patient history. A 17-year-old male was referred for cytogenetic analysis in October 2008 due to a small penis with small testes (the right and left testicular volume of 4 and

Figure 1. Karyotype from a peripheral blood metaphase of the patient showing 46,X,idic(Y)(q11.21).
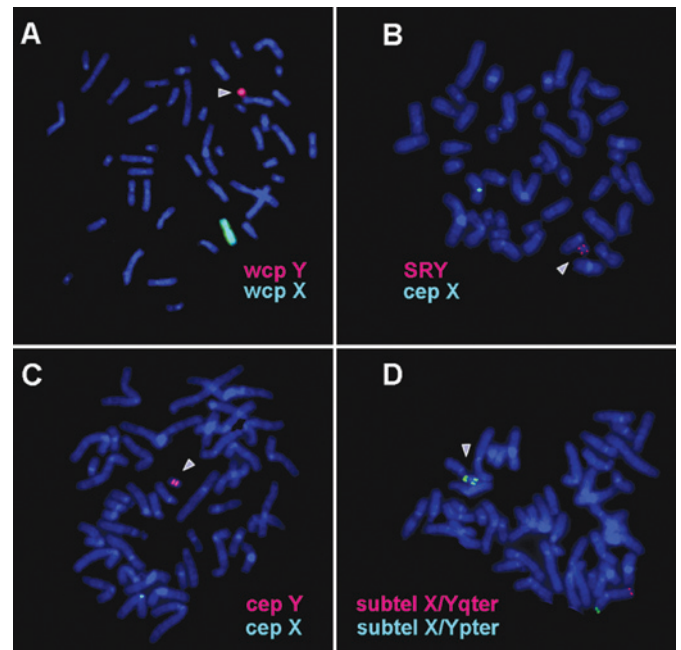

Figure 2. (A) Fluorescence *in situ* hybridization (FISH) using probes for whole painting chromosome X (green) and Y (red) showing one red signal is on the der(Y) and one green signal is on the chromosome X. (B) FISH using probes for the sex-determining region of Y chromosome (SRY) (red) and CEP X (green) showing two red signals is on the der(Y), one green signal is on the chromosome X. (C) FISH using probes for CEP Y (red) and CEP X (green) showing two red signals is on the der(Y), one green signal is on the chromosome X. (D) FISH using probes for ST Xp/Yp (green) and ST Xq/Yq (red) showing two green signals is on the der(Y), one green and one red signals is on the chromosome X.

4.5 ml, respectively). Informed consent was obtained from the patient and ethics approval for the study was provided by the Bio-safety and Bioethics Member Committee/S.A.E.C. The patient's weight was 69 kg, with a height of 170 cm, and he did not show any dysmorphic signs or symptoms. The mother was a 37-year-old healthy female without any signs or symptoms of virilization or drug abuse during pregnancy. The mother's and the father's height were 167 and 184 cm, respectively. No family history of sterility or abortion was reported. Ultrasound of the pelvis area revealed no uterus and ovaries. Laboratory examination showed FSH levels of 2.1 mIU/ml, LH levels of 1.6 mIU/ml and a testosterone concentration of 0.89 ng/dl. Volume semen analysis was 2.5 ml.

Cytogenetic and molecular cytogenetics analysis. Cytogenetic analysis using GTG-banding was performed according to standard procedures (9). A total of 100 metaphases analyzed from stimulated peripheral blood cultures were analyzed. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (10).

Fluorescence *in situ* hybridization (FISH) was carried out on the metaphases using a commercially available probes whole painting chromosome (WCP), LSI SRY, subtelomeric for Xp/Yp and Xq/Yq, centromeric for X and Y (DXZ1; DYZ3) (Abbott Molecular/Vysis, Des Plaines, IL, USA) and Y chromosome-specific multicolor banding (MCB). The procedure was performed as described in a previous study (11). A total of 50-100 metaphase spreads were analyzed. The results were evaluated on a fluorescence microscope (AxioImager.Z1 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (4',6'-diamino-2-phenylindole). Image capturing and processing were carried out using an Isis imaging system (MetaSystems, Altlussheim, Germany) for the evaluation of MCB.

Molecular analysis. Genomic DNA was extracted from peripheral blood using Genequality AZF MX kit (AB Analitica, Italy) according to the manufacturer's instructions. To screen for Y-specific regions, we tested for the presence of 26 Y-DNA loci via a sequence-tagged site (STS) and genes. The STS primers tested were: sY81, sY82, sY84, sY86, DBY, USP9Y (AZFa); sY95; sY117, sY121, sY124, sY125, sY127, sY134,
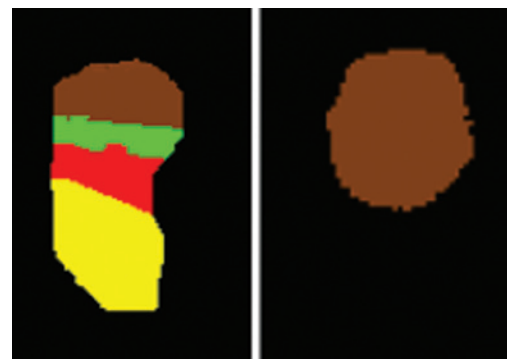

Figure 3. Multicolor banding (MCB) with Y chromosome probe showing idicY(q11.21) (right panel) in comparison to a normal Y chromosome (left panel).

sY142, sY143 (AZFb); sY147, sY149, sY158, sY239, sY242, sY254 (DAZ), sY255 (DAZ), sY283 (AZFc); sY145, sY153 (AZFd). Moreover, sY160 (heterochromatin region) and human zinc-finger protein-encoding genes (ZFX/ZFY) located on the X and Y chromosomes were selected. The ZFX/ZFY and SRY (i.e., STS sY14) acted as internal control primers. Following an initial denaturation step at 94˚C for 5 min, cycle parameters were: 40 cycles at 94˚C for 1 min, 60˚C for 1 min, and 72˚C for 1 min. The program was followed by the final extension step at 72˚C for 5 min. The reaction products were electrophoresed on an ethidium bromide-stained 2.5% agarose-TAE-gel and observed under UV-light.
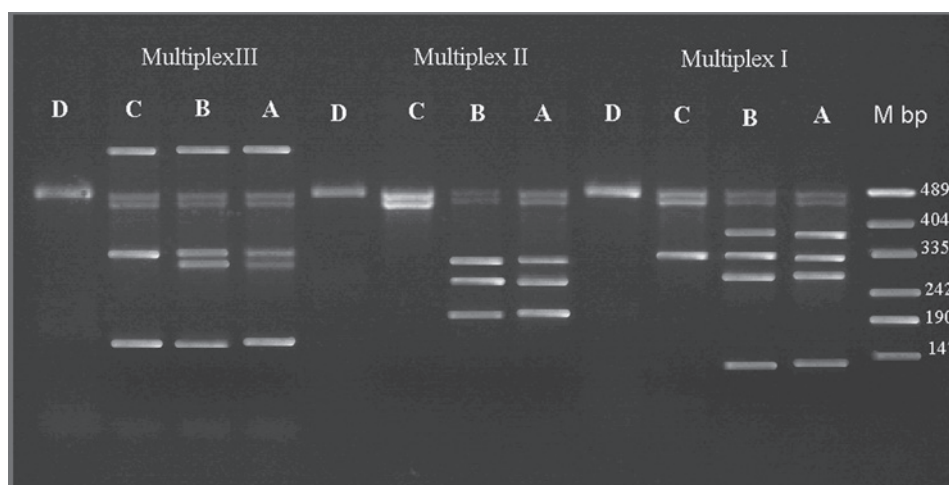
Figure 4. Multiplex PCR for the presence or absence of 26 Y-specific loci were analyzed in the patient with a Y chromosome deletion. Multiplex I: Zinc-finger protein-encoding genes (ZFX/ZFY) (495 bp), sex-determining region of Y chromosome (SRY) (472 bp), sY254 (380 bp), sY86 (320 bp), sY127 (274 bp) and sY255 (120 bp); Multiplex II: ZFX/ZFY (495 bp), SRY (472 bp), sY95 (303 bp), sY117 (262 bp) and sY125 (200 bp); and Multiplex III: DBY (689 bp), ZFX/ZFY (495 bp), SRY (47 bp), sY84 (326 bp), sY134 (301 bp) and DFFRY (155 bp). This patient had seven (sY95, sY117, sY125, sY127, sY134, sY254 and sY255) deletions. Each reaction was compared with a fertile man serving as the control (A and B); C, DNA sample of patient; D, sample from a woman as a negative control; and M, DNA molecular weight marker. Additionally the loci (sY145, sY147, sY149, sY158 and sY283) were deleted (data not shown).

PCR amplification of SRY and sequencing analysis. According to the procedure of Al-Achkar *et al* (12), mutation screening was carried out using direct DNA sequence analysis. The whole coding sequence of the SRY gene was amplified by PCR using the primers previously described (13).

Results

The karyotype determined by GTG-banding was identified as mos 45,X/46,X,+mar/47,XX,+mar (Fig. 1). FISH using a Y chromosome-specific whole chromosome painting probe stained the entire derivative chromosome (Fig. 2A). Findings of dual color FISH experiments (Fig. 2B-D) showed that the chromosome was an isodicentric mainly derived from the short arm of the Y chromosome. Together with the MCB-findings (Fig. 3) the karyotype was obtained was: 45,X[4]/46,X,idic(Y)(q11.21)[95]/47,XX,+idic(Y)(q11.21)[1].

The presence or absence of 26 Y-specific loci was analyzed molecularly. Of the 26 loci, 12 loci were absent in the present case: sY95, sY117, sY125, sY127, sY134, sY145, sY147, sY149, sY158, sY254, sY255 and sY283 (Fig. 4). Thus, the breakpoint of the derivative Y chromosome was determined to be between marker sY84 (13.4 MB) and sY95 (15.1 MB). According to cytogenetics, the breakpoint was in band Yq11.21, located between 12.5 and 14.3 MB. Therefore, the breakpoint is likely to be between 13.4 and 14.3 MB (hg18).

To identify a possible mutation, we analyzed the SRY-specific PCR fragment by DNA sequencing using a normal male as a control. No mutation was exhibited by the patient (data not shown).

Discussion

The most common abnormality in the human Y chromosome is a dicentric derivative present as part of a mosaic karyotype including a 45,X cell line (3). A number of mechanisms of dicentric isochromosome formation have been described

and the most frequent mechanism of this alteration is the sister-chromatid breakage and reunion (14-17). Isodicentric Y chromosomes appear to be formed by a single break in one of the Y chromatids, followed by a fusion of the broken ends of sister chromatids and the loss of the acentric fragment during gametogenesis prior to the formation of spermatids. Such rearrangements are generally unstable and an additional 45,X cell line is frequently present (18). Patients with dicentric isochromosome Y are mosaics without a 46,XY cell line, indicating that gametogenic or early post-zygotic origins are the most frequent (1), and a 45,X cell line is usually found as part of this mosaicism at the same time (1,3). In the present study, the dicentric isochromosome Y was also mosaic but without a normal cell line (1,3).

Although in the literature patients with mosaic karyotypes 45,X/46,X,idic(Y)(q11.21) have frequently been described (http://www.med.uni-jena.de/fish/sSMC/sturner.html), the present patient is to the best of our knowledge the first case with mos 45,X/46,X,idic(Y)(q11.21)/47,XX,+idic(Y)(q11.21) and a Klinefelter syndrome phenotype.

The Klinefelter syndrome is most common, occurring in 1:500 to 1:1000 live births (19). The phenotype of patients is variable such as tall stature, hypogonadism, gynecomastia, small testes, decreased facial hair, small penis, infertility and low testosterone (20). The classic form of Klinefelter syndrome is the polyploidy (extra chromosome) condition of 47,XXY, which characterizes 80% of Klinefelter cases. Another 15% of the cases are mosaic types (46,XY/47,XXY, 46,XY/48,XXYY, 45,X0/46,XY/47,XXY and 46XX/47,XXY). The remaining 5% are XX forms with the SRY gene (testes determining factor) translocated to an X chromosome, Poly X + Y forms, or combined mosaic and poly X + Y forms (21). In the present study, the 47,XX,+idic(Y)(q11.21) condition was present in only 1% of the peripheral blood cells.

The presence of a Y chromosome is associated with a 25-70% risk, the highest risk being associated with mixed gonadal dysgenesis and increasing age. The development of

gonadoblastoma is thought to be less common in patients with idic(Y)(q11.2) (22).

In conclusion, we present a rare case of a clinical presentation of Klinefelter syndrome with only 1% of the peripheral blood cells having the causative two X-chromosomes. We suggest that the patient has more cells with 47,XX,+idic(Y)(q11.21) karyotype in other tissues. This case may be useful to explain similar clinical Klinefelter syndrome cases without typical karyotypic findings in peripheral blood. The break-point of the derivative Y chromosome was also narrowed down to 0.9 MB.

Acknowledgements

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