Clinical and molecular cytogenetic studies in three infertile patients with mosaic rearranged Y chromosomes

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Isodicentrics (idic) are structural anomalies of the Y chromosome associated with a 45,X cell line and a broad spectrum of phenotypes. We characterized the rearranged Y chromosomes from three azoospermic males by fluorescence in-situ hybridization (FISH) and PCR. Chromosome study was performed on lymphocytes and testicular biopsy. FISH analysis and PCR established the degree of mosaicism and analysed specific Y regions. Two patients showed a 45,X/46,X,?idic(Y) karyotype with varying degrees of mosaicism. FISH demonstrated the presence of two centromeres and two SRY regions. In the lymphocytes of the third patient, the presence of a small Y-derived marker was also observed. An additional cell line with two idic(Y) was present in the testicular biopsy of the same patient. PCR showed the breakpoint between SY182 (KALY) and SY121 in Yq11.221-q11.222 region in all the cases. For the evaluation of the mosaicism, different tissues must be investigated. The phenotypical sex depends more on the number of copies of the SRY gene rather than on the percentage of 45,X cells, at least in the gonads. The combined use of classical and molecular cytogenetics is necessary for delineating the chromosome regions involved allowing a better genotype–phenotype correlation.

Key words: dicentric Yp/fluorescence in-situ hybridization/genotype–phenotype correlation/mosaicism/PCR

Introduction

Dicentrics are the most common structural anomalies of the Y chromosome, but very few have been characterized by molecular cytogenetic techniques including fluorescence in-situ hybridization (FISH) and PCR on different tissues (Stuppia et al., 1996; Robinson et al., 1999; Godoy Assumpção et al., 2000; Jakubowski et al., 2000). Structural aberration of Y chromosome is commonly described in association with a 45,X cell line because of the instability of the rearranged chromosome (Hsu, 1994). The phenotypes of patients with 45,X/46,X,der(Y) karyotype range from almost normal males through mixed gonadal dysgenesis to females with Turner phenotype. Mosaicism degree varies between tissues, and it has been reported that phenotypic sex strongly depends on the percentage and distribution of the Y chromosome in the gonads, irrespective of the presence of SRY. Also the structure of the rearranged Y chromosomes plays an indirect role in the phenotypic sex: since in the idicYp the very proximal breakpoint in the q arm makes the chromosome more unstable; this results in a higher percentage of 45,X cell line, leading to a female phenotype (Kelly et al., 1998; Reddy and Sulcova, 1998; Quilter et al., 2002).

In this report, we present the clinical, cytogenetic, FISH and molecular studies of three infertile male carriers of a mosaic karyotype with rearranged Y chromosomes.

Materials and methods

Clinical reports

Patient 1

This patient is a 41-year-old male referred to our Reproductive Medicine Unit for ICSI. Sporadic spermatozoa were found in the first semen analysis in 1988, but normal volume azoospermia was established in four successive samples analysed in another hospital. In 1992, a bilateral testicular biopsy allowed the diagnosis of Sertoli cell-only syndrome (SCOS). Physical examination performed at our hospital showed a height of 171 cm and a weight of 71 kg. The secondary sexual characteristics, epididymides, vasa and penis were normal, while the testes were 5 ml in volume. No ultrasonographic anomalies of the testes and distal seminal ducts were observed. The FSH level was 13.7 IU/l (normal range: 1.3–9.0 IU/l). His medical history was significant for mild hyperprolactinaemia without significant symptoms, and he was successfully treated with bromocriptine. He underwent testicular biopsy with testicular sperm extraction (TESE). Diagnostic testicular biopsy histopathologically confirmed SCOS, while sperm extraction procedure, performed on a second testicular parenchyma specimen (80 mg), revealed the presence of some spermatogenic cells and three mature spermatozoa. Normal sexual activity was reported by his wife.

Patient 2

This patient is a 35-year-old man who presented to our clinic after 8 years of primary infertility. His medical history was significant for
mesangial immunoglobulin IgA–IgG deposition (Berger’s syndrome) associated with light liver insufficiency and hypothyroidism secondary to Hashimoto’s thyroiditis, requiring levothyroxine chronic treatment. Sporadic spermatozoa were found in the first semen specimen, but normal volume azoospermia was later ascertained in three other analyses. High FSH values, 15.2 and 16.1 IU/l, were already detected in 2002 and 2004. Physical examination showed that he was 163 cm in height and 62 kg in weight. Secondary sexual characteristics and penis were normal, while testes were soft and hypotrophic (4 ml on both sides). Epididymides, vasa, seminal vesicles and prostate were normal at the ultrasound examination. Normal sexual activity was reported.

**Patient 3**

The patient, a 31-year-old male, presented with a 4-year history of primary infertility. A large varicocele, normal volume azoospermia and normal FSH were already observed in another hospital. Physical examination showed normal secondary sexual characteristics and a large penis, while the testis volumes, sonographically determined, were 11 ml on the right and 10 ml on the left. Epididymides, vasa, seminal vesicles and prostate were normal. His height was 170 cm and he weighed 85 kg. A semen analysis confirmed azoospermia; LH, testosterone and prolactin were in the normal range, while FSH was 9.2 IU/l. The patient had testicular biopsy with TESE, and pure SCOS was diagnosed histopathologically, and in sperm extraction procedure, no spermatogonial cells were observed. He reported a normal sexual activity.

**Cytogenetic and FISH analyses**

Chromosomal analyses were performed on peripheral blood lymphocytes and tissue cultured from testicular biopsies using standard techniques and QFQ banding (Caspersson et al., 1970; Zackai and Meldman, 1974). FISH studies were performed on blood samples using the LSI SRY/CEPX probes (Abbott-Vysis, Downer Grove, IL, USA) and the CEPX, CEPY, CEP18-Aneuvysion kit (Abbott-Vysis, Downer Grove, IL, USA) according to the manufacturer’s instructions.

**Molecular analysis**

Patient DNA was analysed by PCR using a commercial kit (Promega, Madison, WI, USA) as part of a study to screen Y chromosome micro-deletions in infertile males of couples who have to undergo IVF. DNA was extracted from peripheral blood and analysed, according to the manufacturer’s recommendations. Five Multiplex Master Mixes, with a total of 20 characterized Y-specific primer pairs, are included. Four of the multiplex primer sets contain a control primer pair that amplifies a fragment of the X-linked SMCY locus. One of the multiplex primer sets (Multiplex E Master Mix) contains a control primer pair that amplifies a unique region in both male and female DNA (ZFX/ZFY). Finally, a primer pair that amplifies a region of both male and female DNA (ZFX/ZFY) has been included in Multiplex E Master Mix as a control for the testis-determining factor on the short arm of the Y chromosome to identify the presence of a dicentric Yp chromosome. The molecular study performed by 19 Yq sequence-tagged sites (STS) showed the presence of SY81, SY86, SY84 and SY182 loci, while, starting with SY121 in the AZFb region, the remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in Yq11.221-q11.222 region corresponding to SY121-q11.222 loci in the AZFb region. The molecular study performed by 19 Yq sequence-tagged sites (STS) showed the presence of SY81, SY86, SY84 and SY182 loci, while, starting with SY121 in the AZFb region, the remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in Yq11.221-q11.222 region corresponding to SY121-q11.222 loci in the AZFb region. The remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in Yq11.221-q11.222 region corresponding to SY121-q11.222 loci in the AZFb region.

Figure 1 shows the Y chromosome ideogram and the position of the primers used.

**Results**

The cytogenetic and FISH results are summarized in Table I. In all the three patients, classical cytogenetic, FISH and molecular studies gave the same results for the idic(Y) (Figure 2).

In patient 1, classical cytogenetics performed on peripheral blood lymphocytes showed the presence of two cell lines: 45,X[12]/46.X,idic(Y)(p10)[38]. The same karyotype was observed in cultures from testicular biopsy. FISH characterization of the rearranged chromosome Y demonstrated the presence of double hybridization signal using the Y alpha-satellite (DYZ3) probe and the presence of two SRY regions, confirming the suspicion of a dicentric Yp chromosome. The molecular study performed by 19 Yq sequence-tagged sites (STS) showed the presence of SY81, SY86, SY84 and SY182 loci, while, starting with SY121 in the AZFb region, the remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in Yq11.221-q11.222 loci in the AZFb region. The remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in Yq11.221-q11.222 loci in the AZFb region. The remaining loci tested were deleted. This result allowed us to establish the chromosomal breakpoint in Yq11.221-q11.222 loci in the AZFb region.

Figure 1. Results of molecular analysis, as observed in all three patients, related to Y chromosome ideogram (left) and deletion map (right). The common proximal breakpoint in the three patients was between SY182 and SY121 (bold). Loci order according to UCSC Genome Browser (http://www.genome.ucsc.edu). Deletion map reported by Foote et al.
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FISH analysis with the CEPY probe on interphase nuclei was not reliable because of the difficulty to discriminate between the idic(Y) and the der(Y) signals. In Table II, we report the comparison between the metaphase/interphase FISH results, which shows an inversion of the percentage of cells with the der(Y) or idic(Y) alone. Based on this observation, we decided to consider FISH on nuclei to be not informative and extend the FISH analysis to all the metaphases scorable. Also for this patient, the molecular result is the same as in the previous cases (Figure 1).

**Table I.** Classical cytogenetic and fluorescence in-situ hybridization (FISH) results

<table>
<thead>
<tr>
<th>Patients</th>
<th>Peripheral blood</th>
<th>Testicular biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytogenetic results (%)</td>
<td>FISH results (%)</td>
</tr>
<tr>
<td></td>
<td>46,X,idic(Y)(p10)[38] (76)</td>
<td>46,X,idic(Y)(q11)(DYZ3++)(SRY++) [152] (76)</td>
</tr>
<tr>
<td>2</td>
<td>45,X[17] (34)</td>
<td>45,X[40] (20)</td>
</tr>
<tr>
<td></td>
<td>46,X:idic(Y)(p10) [33] (66)</td>
<td>46,X:idic(Y)(q11)(DYZ3++)(SRY++) [160] (80)</td>
</tr>
<tr>
<td>3</td>
<td>45,X[33] (66)</td>
<td>45,X[56] (56)</td>
</tr>
</tbody>
</table>

*For patients 1 and 2, the values indicate interphase FISH results (%) and for patient 3, the values indicate metaphase FISH results (%).

**Figure 2.** Appearance of the isodicentric Yp [idic(Yp)] and small Y-derived marker (mar) observed in patient 3 by Q-banding (A), dual-colour fluorescence in-situ hybridization using CEPY/CEPX (B) and LSI SRY/CEPX (C). The idicY chromosome in the two other patients showed identical results with these probes.

**Discussion**

Isodicentric Y chromosomes, either idic(Yp) or idic(Yq), are common structural rearrangement of the Y chromosome in infertile males, especially those with azoospermia (Van Assche et al., 1996); nevertheless, many individuals with mosaic idic(Yp) are phenotypically females (Robinson et al., 1999). In fact, such rearrangements are unstable and an additional 45,X cell line is often present (Hsu, 1994). It has been suggested that the percentage of 45,X cells in the urogenital ridge plays a main role in sex determination and differentiation of the gonads, as well as the structure of the dicentric Y chromosome. Previous cytogenetic studies demonstrated that about 4–6.2% of patients with Y chromosome mosaicism are phenotypically females regardless of the presence of SRY (Ostrer and Clayton, 1989; Jacobs et al., 1990; Tuck-Muller et al., 1995; Kelly et al., 1998; Robinson et al., 1999; Godoy Assumpção et al., 2000; Quilter et al., 2002). Quilter et al. (2002), after using standard cytogenetic methods and FISH on peripheral blood, buccal and gonadal tissues, reported a range of 36–95% of metaphases with a ?derY chromosome in infertile men whereas 3–8% of metaphases in female patients with Turner stigmata. Interphase FISH analysis showed a higher percentage of the Y-bearing cells in buccal and gonadal tissues in both females and males but never higher than 35% in Turner patients. Another study reports on three patient carriers of idicYp identified in peripheral blood and urinary tract cells. One of the patients had the highest level of cells with idi-Yp and would be expected to present male development instead of ambiguous genitalia (Stuppia et al., 1996). Since no mutation on SRY was found, one possible explanation of this finding could be that in the gonads, not investigated in the study, more cells with the rearranged Y were present.

**Table II.** Comparison between the metaphase and interphase fluorescence in-situ hybridization (FISH) scoring on 100 cells in patient 3

<table>
<thead>
<tr>
<th>Cytogenetic results</th>
<th>Metaphase FISH results [number of cells] (%)</th>
<th>Interphase FISH results [number of cells] (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45,X</td>
<td>[56] (56)</td>
<td>[110] (55)</td>
</tr>
<tr>
<td>46,X,?idic(Y)(p10),ish idic(Y)(q11)(DYZ3++)(SRY++)</td>
<td>[26] (26)</td>
<td>[22] (11)</td>
</tr>
<tr>
<td>46,X,+mar.ish der(Y)(q10)(DYZ3+)</td>
<td>[10] (10)</td>
<td>[42] (21)</td>
</tr>
<tr>
<td>47,X,?idic(Y)(p10),+mar.ish idic(Y)(q11)(DYZ3++)(SRY++), der(Y)(q11)(DYZ3+)</td>
<td>[8] (8)</td>
<td>[26] (13)</td>
</tr>
</tbody>
</table>

The percentage of cells with idic(Y) and mar alone are not comparable (in bold).
Azoospermic males with der(Y) chromosomes

Our study suggests that the phenotypic sex depends on the percentage of cells with the SRY gene in the gonads and indicates that a cytogenetic study performed on the blood cells only is not informative. Patient 3 had 24% of the blood cells bearing the SRY gene, considering both cell lines with the idic(Y) and idic(Y)+mar, but had 48% of the cells in the gonads bearing the SRY gene since we observed an additional cell line with two idic(Y). To our knowledge, patient 3 is the only male patient with the lowest level of rearranged Y-bearing cells in the gonads and no Turner features reported. This could be explained by the presence of more copies of the SRY gene, suggesting a possible dosage effect.

The common proximal breakpoint in our three patients, between STS SY182 and SY121 in region 5 of the deletion map (Foote et al., 1992), does not seem to interfere with the stability of the rearranged Y, leading to a higher percentage of 45,X cells and a female phenotype, as was previously reported (Jakubowski et al., 2000).

In conclusion, our study confirms the necessity to cytogenetically investigate the gonadal tissue in patient carriers of rearranged Y chromosome(s) since the phenotypic sex seems to be determined by the percentage of the 45,X versus SRY-bearing cells. FISH analysis is a powerful tool in defining the Y rearrangement and extending the number of cells investigated, allowing a more precise evaluation of the mosaicism. Molecular study by PCR technique is also needed to better characterize the chromosomal breakpoints and the deletion interval.

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References


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