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Mitotic and meiotic behaviour of a naturally transmitted ring Y chromosome: reproductive risk evaluation

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BACKGROUND: The mitotic and meiotic behaviour of a transmitted ring Y [r(Y)] chromosome from a father to his Klinefelter syndrome (KS) son, and the mechanism of ring formation are analysed herein. To our knowledge, this is the first reported case of natural transmission of an r(Y). METHODS and RESULTS: Amplification of X chromosome polymorphisms by PCR showed that the KS was of paternal origin. G-banding and fluorescence *in situ* hybridization (FISH) studies revealed a similar percentage of mosaicism in father and son by mitotic loss of r(Y). SRY gene and Y marker amplification by PCR, FISH with subtelomeric probes for Xp/Yp and Xq/Yq, and comparative genomic hybridization (CGH) analyses indicated the intactness of the Y chromosome from SRY to subtelomere Yq. FISH analysis of sperm from the father showed significantly higher frequencies ($P < 0.005$) for diploidy and for 6, 13, 18, 21, 22, XX, XY disomies than those observed in control donors. CONCLUSIONS: An r(Y) with low material loss can be naturally transmitted, showing similar mitotic behaviour in the offspring. The presence of an r(Y) chromosome in germinal cells increased the risk of fathering offspring with numerical abnormalities, even for chromosomes not involved in the arrangement.

Key words: aneuploidy/inherited ring/Klinefelter syndrome/ring Y chromosome/sperm

Introduction

The frequency of ring chromosomes in clinically detected conceptions is 1/25 000 (Jacobs, 1981) and has been reported for all human chromosomes (Schinzel, 2001). The majority of ring chromosomes seem to be the result of two terminal breaks in both chromosome arms followed by fusion of the broken ends leading to the loss of genetic material (Miller and Therman, 2001). However, other mechanisms of ring formation have been described: the union of a broken chromosome end with the opposite telomere region (Henegariu *et al.*, 1997a); subtelomeric sequence fusion (Vermeesch *et al.*, 2002) or telomere–telomere fusion with no loss of genetic material (Henegariu *et al.*, 1997a; Sigurdardottir *et al.*, 1999).

Generally, the patients with a ring chromosome have a second monosomic cell line because of the instability of the ring chromosome during mitosis produced by the occurrence of sister chromatid exchanges (SCE). These SCE could result in dicentric ring formation that undergo bridge–breakage–fusion–bridge cycles that finally will be lost. The result is a monosomic cell which may or may not be viable (Miller and Therman, 2001).

Up to now, many cases with a ring Y chromosome have been published, but only some of them have analysed the ring chromosome by molecular techniques (Kozma *et al.*, 1988; Wegner *et al.*, 1992; Pezzolo *et al.*, 1993a; Henegariu *et al.*, 1997a,b; Bofinger *et al.*, 1999; Tzancheva *et al.*, 1999; Blanco *et al.*, 2003). Most patients present a mosaic 46,X,r(Y)/45,X karyotype, with phenotypes ranging from Turner syndrome to male external genitalia with hypogonadism or azoospermia (reviewed by Daniel, 1985; Hsu *et al.*, 1994). This phenotype variability depends on the percentage of the monosomic cell line in the different tissues and on the regions deleted during the ring Y formation. Moreover, the coexistence of different derivatives of the original ring could make these phenotypes more variable (Pezzolo *et al.*, 1993a; Henegariu *et al.*, 1997b).

Most ring chromosomes arise *de novo* and $\leq 1\%$ of all ring chromosomes are inherited (revised by Kosztolányi *et al.*, 1991). Ring chromosomes most frequently transmitted are chromosomes 20, 21 and 22 (Palmer *et al.*, 1977; Stoll and Roth, 1983; Hertz, 1987; Back *et al.*, 1989; Kennerknecht *et al.*, 1990). There is only one case reported in the literature of a ring Y chromosome transmitted by ICSI (Bofinger *et al.*, 1999).

Despite meiotic behaviour studies of Y ring chromosomes allowing for both the elucidation of the infertility bases and the risk of fathering aneuploid offspring, only two cases have been published in infertile patients. One of them was carried out in spermatocyte metaphases from testicular biopsies using conventional cytogenetic techniques (Chandley and Edmond, 1971). The second one analysed sperm nuclei

and meiotic cells from one semen sample by the multicolour fluorescence *in situ* hybridization (FISH) technique (Blanco *et al.*, 2003).

The case of a naturally inherited ring Y [r(Y)] chromosome from a father with karyotype 46,X,r(Y)/45,X to his Klinefelter syndrome (KS) son with karyotype 47,XX,r(Y)/46,XX is reported in this work for the first time.

The aims of the present study were to investigate: (i) the mitotic behaviour of the ring Y chromosome in both father and son; (ii) the mechanism of formation of the r(Y); and (iii) the meiotic behaviour of the ring Y chromosome in sperm from the father and his reproductive risk.

Materials and methods

Clinical report

A couple was referred to our laboratory because of an abnormal karyotype obtained by amniocentesis at 16 weeks of gestation following an altered second trimester maternal serum screening with a Down's syndrome risk estimation of 1:95 [alpha-fetoprotein: 0.67 multiples of the median (MoM); free β-hCG: 2.68 MoM]. Cytogenetic analysis of cultured amniotic fluid cells after G-banding revealed a 47,XX,r(Y)/46,XX karyotype in the fetus (Figure 1). It was the first pregnancy with no previous miscarriages. The mother, a 36-year old woman, presented a normal karyotype. The father, a healthy man aged 33 years, 169 cm tall and a normal male phenotype, had a mosaic 46,X,r(Y)/45,X karyotype (Figure 1). Semen analysis of the father showed oligozoospermia (4.5×10^6 sperm/ml) (World Health Organization, 1992). The child was born after 39.4 weeks of uncomplicated gestation and presented a normal physical examination with normal external genitalia. His birthweight was 3300 g and length at birth 49.5 cm. At 3 years of age the patient was 96 cm tall and weighed 14 500 g. On clinical examination, internal genitalia and development progress were normal. The intelligence of both father and son was apparently normal (IQ not analysed). All donors gave informed consent prior to the study, which was approved by our institutional Ethics Committee.

Lymphocytes and buccal mucosa preparations

Metaphases obtained from peripheral blood lymphocytes were analysed by G-banding and multicolour FISH. Interphase lymphocytes from peripheral blood and endothelial cells from buccal mucosa were also obtained. Metaphase and interphase cells were treated with 0.075 mol/l KCl and nuclei were fixed in Carnoy solution (3 methanol:1 acetic acid). All slides were stored at −20°C until G-banding or FISH were performed. Prior to hybridization, buccal mucosa cell preparations were treated with 60% acetic acid.

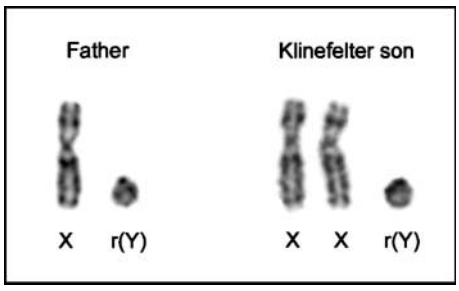

Figure 1. G-banded partial karyotypes showing the sex chromosomes of the father and his Klinefelter son.

Sperm preparations

Semen samples from the father and from eight healthy donors were obtained. Samples were collected and fixed as described (Bosch *et al.*, 2001). Briefly, samples were washed three times in 0.075 mol/l KCl, fixed in methanol:acetic acid (3:1) and smeared onto clean slides. Slides obtained were stored at −20°C until FISH was performed.

FISH analyses

FISH analyses were performed as previously described by Bosch *et al.* (2001). Slides were washed in a standard 2×SSC solution, dehydrated in an ethanol series and denatured for 3–5 min in a 70% formamide solution at 73°C. Probes were denatured at 73°C for 5 min and slides were hybridized in a dark chamber for 12–72 h at 37°C. DAPI-III counterstain (Vysis Inc.) and antifade were applied to the slides prior to observation. Sperm nuclei decondensation was carried out by slide incubation in a solution of 5 mmol/l dithiothreitol at 37°C for 8–15 min before denaturation. For subtelomeric probes, slides were treated with pepsin and postfixed with 1% formaldehyde solution prior to hybridization.

Probes

Centromeric probes for chromosomes 6 (1:1 mix CEP6-Spectrum green and CEP6-Spectrum orange; Vysis Inc.), X (CEPX-Spectrum green; Vysis Inc.), Y (CEPY-Spectrum orange; Vysis Inc. and CEPY-Spectrum green; Oncor Inc.) and 18 (CEP18-Spectrum aqua; Vysis Inc.), locus-specific probes for chromosomes 21 (LSI21-Spectrum orange, loci q22.14-q22.3; Vysis Inc.), 13 (LSI13-Spectrum orange, locus RB-1; Vysis Inc.) and 22 (LSI22-Spectrum green, locus bcr; Vysis Inc.), a satellite III DNA probe for the Y chromosome (Y satIII-Spectrum aqua; Vysis Inc.) and, finally, TelVysion telomeric probes Xp/Yp (Spectrum green) and Xq/Yq (Spectrum orange) (Vysis Inc.) were used for the FISH analyses (Figure 2). Probes used in multicolour FISH in the different cell phases and tissues are shown in Table I.

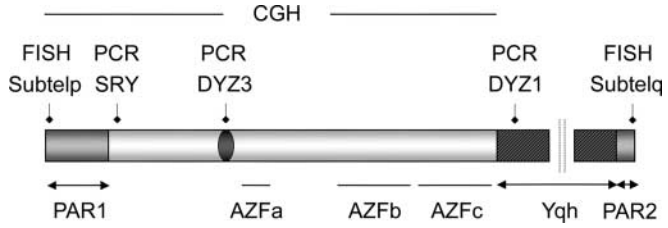

Figure 2. Chromosomal location of gene, regions, probes, and markers on the Y chromosome and the techniques for their detection used in the present study.

Table I. Probes used in multicolour FISH analyses

Cell and phase types	Probes	Type of study
Metaphase lymphocytes	6, X, Y	Mosaicism and dic r(Y) detection
	Tel Xp/Yp, Tel Xq/Yq	r(Y) subtelomere detection
Interphase lymphocytes	6, X, Y	Mosaicism detection
Interphase buccal mucosa	6, X, Y	Mosaicism detection
Sperm nuclei	6, 13, 18, 21, 22, X, Y	Numerical abnormality detection

Data collection and scoring criteria

All slides scored had a hybridization efficiency of ≥99%. Two signals of the same colour were scored as two copies of the chromosome when they were comparable in brightness and size, and separated from each other by a distance greater than the diameter of each signal. Nullisomy was considered when one of the signals was missing, and the nucleus contained one signal for the other chromosomes. All slides were scored by the same person, and ambiguous signals were examined, at least, by a second observer. For subtelomeric probes, cells were scored only when Xp and Xq hybridization signals were present. In sperm analysis, only intact and non-overlapped sperm nuclei, identified as decondensed sperm by the presence of a tail, were evaluated. We considered as diploid those sperm with two signals for each of the chromosomes studied. Scoring of sperm nuclei was blind and slide identification was unavailable to the scorer until data collection was completed. Slides were analysed on an Olympus AX70 epifluorescence microscope equipped with a triple-band pass filter for FITC/Texas Red/DAPI and a single-band pass filter for FITC, Texas Red, Aqua and DAPI. The images were analysed with a Cytovision system (Applied Imaging, UK).

CGH analysis

DNA was extracted from peripheral blood samples of the father according to standard procedures. Comparative genomic hybridization (CGH) was carried out to identify the size of terminal deletions in the ring Y chromosome. DNA labelling, hybridization and detection were performed as described by Hernando *et al.* (2002). The red-labelled paternal DNA and the green-labelled control DNA were hybridized on normal female metaphases. Slides were analysed using a Cytovision Ultra Workstation (Applied Imaging). Fluorescent ratio values of CGH >1.25 and <0.75 were considered to represent chromosomal gain and loss respectively.

DNA analysis

To establish the parental origin of the extra X chromosome in the KS patient, eight microsatellite DNA markers along the X chromosome length were analysed: DXS1283E, DysII, DMD49, DXS991, AR, DXS101, DXS1192, DXS8377 (details about these markers can be downloaded at http://www.gdb.org). DNA from lymphocytes of the family (mother, father and son) was obtained using a standard salt procedure. Amplified products were separated on a 6% acrylamide:bisacrylamide (19:1) gel. PCR amplification was carried out to ascertain the presence of the sex-determining region Y (SRY) gene (sY14/Yp11.3), DYZ3 (sY78/Ycen) and DYZ1 (sY160/Yq12) chromosome Y markers (Figure 2). Internal controls were simultaneously amplified with SRY (DysII located in chromosome X) and DYZ3 and DYZ1 (IR5 located in chromosome 19). DNA amplifications were performed in a final reaction volume of 50 μl containing 1% of standard PCR buffer (Ecogen), 250 μmol/l of each dNTP (Amersham Pharmacia Biotech Inc), 0.8 μmol/l of each primer (Research Genetics), 1.5 mmol/l of $MgCl_2$ (Ecogen), 0.5 IU of Eco-taq Polymerase (Ecogen) and 0.5–0.8 μg of DNA in a Perkin Elmer thermal cycler for 24–30 cycles. DNA amplifications for the Y chromosome were accomplished as explained with the addition of 0.8 mmol/l of the second primer.

Statistical analysis

To investigate whether there were statistically significant differences between the cell-cycle phase studied, tissue analysed and technique used, a χ^2-test was performed. When the χ^2-test was not applicable, Fisher's test was used. A likelihood ratio χ^2-test was used for the analysis of the homogeneity in sperm analysis of the control group. To compare the results from the control group and the KS father, Student's *t*-test was performed. When this test was not applicable due to the heterogeneity of the group, χ^2-test was applied.

Results

Parental origin of the Klinefelter syndrome

Six of the eight X chromosome polymorphisms amplified by PCR were informative (DXS1283E, DysII, DMD49, AR, DXS101 and DXS8377) and showed that the supernumerary X chromosome present in the KS patient was of paternal origin.

Cytogenetic and FISH analysis in somatic cells

A total of 2628 cells were analysed using G-banding and multicolour FISH in father and son: 1490 metaphase and interphase lymphocytes (a minimum of 100 metaphases and 500 interphase nuclei scored per individual) and 1138 interphase buccal mucosa cells (Table II). G-banding and FISH analyses were used to determine the proportion of mosaicism in metaphase lymphocytes. Multicolour FISH was also used to compare the percentages of each cell line in the different cell-cycle phases and tissues studied in father and son (Figure 3).

Table II. Mitotic behaviour of the ring in Klinefelter syndrome (KS) father and KS patient in different cell cycle phases and tissues using both G-banding and multicolour fluorescence *in situ* hybridization (FISH)

Tissue phases	Technique used	Father			Klinefelter son		
		No. of cells analysed	46,X,r(Y)	45,X	No. of cells analysed	47,XX,r(Y)	46,XX
Metaphases							
Lymphocytes	G-banding	110	67.3	27.3	110	74.6	22.7
Lymphocytes	FISH	114	71.9	25.4[a]	112	69.6	29.5[a]
Interphases							
Lymphocytes	FISH	520	86.9	12.3	524	78.4	20.0[b,c]
Buccal mucosa	FISH	540	84.4	13.0	598	84.6	13.0

[a]Increased frequency in aneuploid cell line in metaphase lymphocytes compared with interphase lymphocytes in the same individual ($P < 0.05$).
[b]Increased frequency in interphase lymphocytes compared with interphase buccal mucosa cells in the proband ($P < 0.05$).
[c]Increased frequency in interphase lymphocytes in the proband compared with interphase lymphocytes in the father ($P < 0.05$).

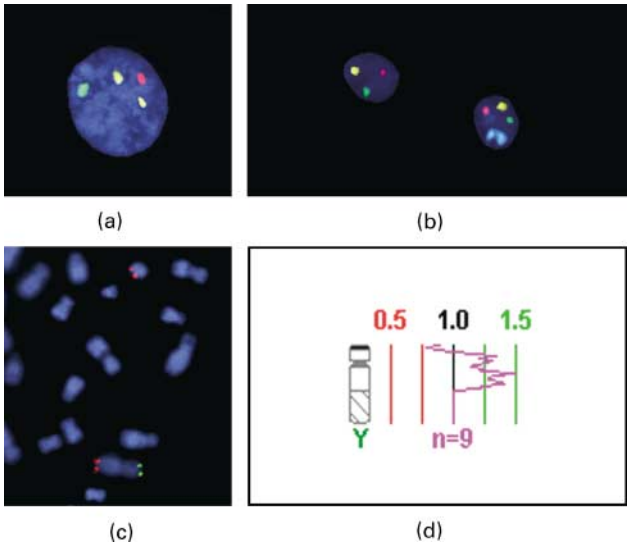

Figure 3. (**a**) Normal buccal mucosa cell of the father with signals for 6 (yellow), X (green) and Y (red) chromosomes. (**b**) Two sperm nuclei from the father with signals for 6 (yellow), 21 (red), X (green) and Y (blue) chromosomes. On the right, a disomic XY sperm. (**c**) A partial lymphocyte metaphase of the father with signals for subtelomere regions for Xp/Yp (green) and Xq/Yq (red). The X chromosome presents both subtelomere regions, whereas the r(Y) chromosome only presents a signal for the Yq subtelomere region. (**d**) Profile of the ring Y chromosome after CGH (DNA was hybridized on normal female metaphases) showing the Y chromosome material present in the r(Y) from the father.

Using G-banding, we found two different cell lines per individual: 46,X,r(Y)[74]/45,X[30] in the father and 47,XX,r(Y)[82]/46,XX[25] in the son. The percentages obtained for the two cell lines were similar when comparing father versus son. There were significant differences in each individual for the frequency of each cell line depending on the cell phase studied (father, $P < 0.005$; son, $P < 0.05$). Differences depending on the tissue analysed were also detected in the son ($P < 0.05$) (see Table II). A significant increase in the loss of the ring Y chromosome in interphase lymphocytes was observed in the son ($P < 0.05$).

In interphase cells analysed by FISH, it was not possible to distinguish between a dicentric r(Y) or two r(Y). In metaphase lymphocytes analysed by G-banding and FISH, a dicentric r(Y) in the father (five cells and three cells respectively) and in the son (three cells and one cell respectively) was found. We also detected two r(Y) in one metaphase lymphocyte (1/110 cells) analysed by G-banding in the father.

FISH with specific subtelomeric probes for sex chromosomes demonstrated the presence of the Yq subtelomere and the absence of the Yp subtelomere in the r(Y) in both father and son (see Figure 3).

Molecular analysis by PCR

Positive amplification of gene SRY and markers DYZ3 and DYZ1 by PCR proved their presence on the r(Y) in both father and son.

CGH analysis

CGH was performed on normal female metaphases to identify the deleted Yp region on the ring Y chromosome. The CGH profile showed the presence of the regions from Yp11.3 to Yq11.2 (Figure 3).

FISH in sperm

A total of 187 126 sperm nuclei were analysed, with a minimum of 10 000 sperm nuclei scored for each hybridization and individual. The results obtained are shown in Table III. A significant increase ($P < 0.005$) of disomy for chromosomes 6, 13, 18, 21, 22, XX, XY and for diploidy were found in the father, compared with control donors (Figure 3). The overall incidence of aneuploidy for all chromosomes studied was statistically higher ($P < 0.005$) in sperm from the father (12.37%) than in the control donors' mean, (1.07%). The X-bearing sperm (58.34%) showed a statistically significant increase ($P < 0.05$) versus Y-bearing sperm (41.66%).

Discussion

Mitotic and meiotic behaviour of a transmitted ring Y chromosome and the mechanism involved in its formation has been analysed by cytogenetics and molecular techniques. To our knowledge, this is the first reported case of a naturally transmitted ring Y chromosome.

Mitotic behaviour of the ring Y chromosome

The mitotic behaviour of the r(Y) in both father and son was similar, showing two cell lines in all the tissues analysed as a consequence of the loss of r(Y) chromosome. The only difference observed between them was a significant increase in r(Y) loss in interphase lymphocytes ($P < 0.05$) in the son. These results could be explained by a higher mitotic instability of the ring in the son due to the presence of two X chromosomes in his karyotype [47,XX,r(Y)/46,XX]. According to several authors, the percentage of the aneuploid cell line would be determinant for a patient carrying a ring chromosome to assess his fertility and phenotype (Wegner *et al.*, 1992; Pezzolo *et al.*, 1993a; Hsu *et al.*, 1994; Henegariu *et al.*, 1997a; Tzancheva *et al.*, 1999). The frequencies of the cell line without the r(Y) in father and son in interphase cells ranged from 12 to 20%, corresponding to the lower percentages obtained by other authors in similar studies (ranging from 5 to 85%) (Chandley and Edmond, 1971; Maeda *et al.*, 1976; Taillemite *et al.*, 1978; Steinbach *et al.*, 1979; Kosztolányi, 1988; Micic *et al.*, 1990; Wegner *et al.*, 1992; Hsu, 1994; Henegariu *et al.*, 1997a; Bofinger *et al.*, 1999; Tzancheva *et al.*, 1999; Blanco *et al.*, 2003).

Both individuals presented an increased loss of the ring Y chromosome in metaphase lymphocytes ($P < 0.05$) versus interphase cells. It has been previously described that colcemid treatment during slide preparation could contribute to artefactual loss of chromosomes due to the absence of the mitotic spindle fibres (Márquez *et al.*, 1996). The different percentages of the 46,XX cell line observed in the son, depending on the tissue studied ($P < 0.05$), have already been described by Henegariu *et al.* (1997a).

Table III. Numerical abnormalities in sperm from the father and the control donors analysed using multicolour FISH

Control donors	Age (years)	Disomy (%)								Diploidy (%)
		6	13	18	21	22	XX	YY	XY	
C1	42	0.10	0.05	0.03	0.33	0.03	0.12	0.04	0.34	0.20
C2	33	0.03	0.05	0.02	0.04	0.03	0.08	0.05	0.19	0.16
C3	38	0.01	0.06	0.04	0.24	0.05	0.08	0.06	0.23	0.18
C4	40	0.03	0.04	0.03	0.16	0.05	0.10	0.04	0.33	0.26
C5	41	0.07	0.06	0.04	0.46	0.02	0.09	0.12	0.26	0.22
C6	48	0.05	0.05	0.03	0.40	0.04	0.14	0.07	0.19	0.20
C7	47	0.06	0.05	0.03	0.27	0.03	0.08	0.08	0.37	0.24
C8	58	0.03	0.08	0.04	0.17	0.07	0.06	0.08	0.29	0.15
Control mean	43.4	0.05	0.05	0.03	0.27	0.04	0.09	0.07	0.27	0.20
KS father	33	0.25[a]	0.50[a]	0.25[a]	0.85[b]	0.45[a]	0.22[a]	0.06	9.32[a]	0.83[a]

[a]Increased frequency compared with the control mean ($P < 0.0001$).
[b]Increased frequency compared with the control mean ($P < 0.005$).
KS = Klinefelter syndrome.

The percentage of dicentric r(Y) found in both father and son (0.89–4.5%) is within the range described by other authors for this chromosome (1.3–18%) (Chandley and Edmond, 1971; Kosztolányi *et al.*, 1988; Micic *et al.*, 1990; Blanco *et al.*, 2003). The low frequency of dicentric r(Y) present in both cases seems to reflect the small number of SCE which occurred in the ring and, thus, its low instability during mitosis.

Mechanism of ring Y chromosome formation

To establish the mechanism of ring formation in father and son, cytogenetic and molecular analyses have been performed. PCR, FISH and CGH results have demonstrated that r(Y) was present from Yp11.3 (above SRY gene) to the Yq subtelomere (see Figure 2). These results are consistent with the loss of part, if not all, of the PAR1 region (starting at Yp11.32).

There are two possible mechanisms of formation for the r(Y) studied. The first one is in agreement with the generally accepted model of ring formation requiring two terminal deletions in both chromosome arms (Micic *et al.*, 1990; Wegner *et al.*, 1992; McGinnis *et al.*, 1992; Pezzolo *et al.*, 1993b; Conte *et al.*, 1995; Sigurdardottir *et al.*, 1999; Tzancheva *et al.*, 1999). The two breakpoints were localized: one on the Yp arm (between Yp11.3 and the subtelomere region) and a second one on the Yq arm (between the subtelomeric region and the telomere). The second possible mechanism, which has been previously described by other authors (Conte *et al.*, 1997; Henegariu *et al.*, 1997a), implies a breakpoint on the Yp arm and fusion with the opposite telomere region.

The normal stature of the father (169 cm) suggests that the genetic material loss implicated in ring Y formation may not have included the SHOX/PHOG locus localized in the PAR1 region of Yp (Yp11.3). These data seem to reflect a partial loss of the PAR1 region instead of a total loss on the r(Y).

Meiotic chromosome segregation in sperm

To assess the possible association between the Y ring presence and abnormal meiotic chromosome segregation, the frequencies of numerical chromosome abnormalities in sperm from the father with those observed in eight control donors, using multicolour FISH, were compared. The father showed a statistically significant increase in the percentage of diploidy, disomy for all autosomes analysed (6, 13, 18, 21, 22), and for XX and XY disomy. Only one similar study has been carried out in an infertile man with a 45,X/46,X,r(Y)/46,X,dicr(Y) karyotype in few sperm because the patient had severe oligozoospermia (Blanco *et al.*, 2003). The authors also observed an increased incidence for diploidy and for 21 and XY disomies, but not for 13 and 18 disomies, probably due to the low number of sperm analysed (<50 sperm).

The high percentage of XY disomy found in the father (9.32 versus 0.27% in controls) ($P < 0.005$) could be the result of the partial lack of PAR1 in the ring and it could explain the paternal origin of the extra X chromosome present in the KS son. Diminished recombination in this region is associated with meiotic non-disjunction of the XY pair during meiosis I (Hassold *et al.*, 1991). In fact, meiotic studies in three patients with a ring chromosome demonstrated unpaired sex chromosomes from 80 to 100% in spermatocyte I cells (Chandley and Edmond, 1971; Micic *et al.*, 1990; Blanco *et al.*, 2003). Recently, an increased frequency of XY sperm ($P < 0.02$) has also been found in fathers of paternal KS cases compared with that observed in fathers of maternal KS cases. The authors explain this increase, in part, because of paternal age (Eskenazi *et al.*, 2002).

The statistically significant increase in X-bearing versus Y-bearing sperm observed in the father by us, and in an infertile man with a r(Y) by other authors (Blanco *et al.*, 2003), could be explained by the loss of r(Y) during meiosis.

The increased disomy observed for all autosomes analysed and XX disomy in sperm from the father could be the product of a generalized susceptibility to non-disjunction in this individual due to the presence of r(Y). An interchromosomal effect has been described due to the presence of a ring chromosome in *Saccharomices cerevisae* (Flatters *et al.*, 1995) and in humans (Chandley and Edmond, 1971; Roeder, 1997; Blanco *et al.*, 2003). The presence of a ring chromosome may produce segregation errors of other chromosomes due to mechanical problems (Flatters *et al.*, 1995) or to the presence of one impaired bivalent (Chandley and Edmond,

1971; Roeder, 1997). More recently, studies in fathers of Turner and Down's syndrome children of paternal origin (Soares *et al.*, 2001a,b) have described an increased frequency of aneuploid sperm not restricted to the chromosome pair implicated in the aneuploid child. The authors suggested the possibility of a generalized susceptibility to non-disjunction due to disruption of the synaptic process.

Ring Y chromosomes and fertility

Sex determination and spermatogenesis are the most important functions of the Y chromosome, and normal male development in humans depends on its presence. All men with a ring Y chromosome studied so far have been infertile patients (Chandley and Edmond, 1971; Maeda *et al.*, 1976; Kozma *et al.*, 1988; Micic *et al.*, 1990; Tzancheva *et al.*, 1999; Blanco *et al.*, 2003). Infertility is the main effect of an r(Y) chromosome due to the loss of Y genes directly implicated in spermatogenesis, the presence of a 45,X cell line or the lack of sex chromosome pairing during meiosis.

The presence of the SRY gene and the intactness of the Yq arm were already expected in the father and son because of their male phenotype. The presence of Y genes implicated in spermatogenesis (regions AZFa, AZFb and AZFc) (reviewed by Foresta *et al.*, 2001; Lahn *et al.*, 2001) and the low proportion of 45,X cells in the father could explain his normal male phenotype. Despite intactness of the Yq arm, the father presented a moderate oligozoospermia which could be explained by an arrest of XO spermatocytes I during meiosis (Chandley and Edmond, 1971; Micic *et al.*, 1990; Blanco *et al.*, 2003). Previously, a correlation between the absence of pairing and spermatogenesis arrest was hypothesized by Miklos (1974). More recently, male individuals lacking XY pairing are known to be sterile because a reduced recombination frequency leads to incorrect segregation (Hassold *et al.*, 1991; Rappold *et al.*, 1993). In our case, the father would probably have been referred to ICSI if his moderate oligozoospermia had been detected previously. Only one case of a ring Y chromosome transmission has been reported (Bofinger *et al.*, 1999) from a father with a 46,X,r(Y)/45,X karyotype to his son achieved by ICSI because of severe oligozoospermia. Unfortunately, in this report the numerical abnormalities in sperm from the father or the regions deleted due to the formation of the ring were not analysed.

The overall frequency of numerical chromosome abnormalities in the KS father was >10-fold higher than those observed in control individuals (12.7% versus 1.1%), including numerical abnormalities for other chromosome pairs not involved in the rearrangement. This generalized predisposition to meiotic non-disjunction could be even higher if more chromosomes were analysed.

In conclusion, an r(Y) with low material loss on the Yp arm can be naturally transmitted, and its mitotic behaviour seems to be similar in the offspring. The intactness of the Yq arm, the low percentage of r(Y) loss, and the presence of the SRY gene are all in agreement with the male phenotype shown by father and son. The increased incidence of sperm with numerical chromosomal abnormalities found in the father could be explained mainly by the partial loss of the PAR1 region and by an interchromosomal effect because of the presence of the ring in germinal cells. The presence of a ring Y chromosome in germinal cells increased the risk of fathering offspring with numerical abnormalities even for chromosomes not involved in the arrangement. These findings may also be associated with an increased production of clinically undetected pregnancy losses that could explain some cases of idiopathic infertility.

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