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ARTICLE

Sperm FISH analysis of a 46,XY,t(3;6)(p24;p21.2), inv (8)(p11;2q21.2) double chromosomal rearrangement

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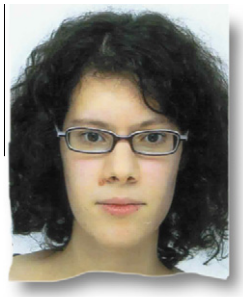

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Abstract A complex chromosome rearrangement (CCR) can be defined as a structural chromosomal aberration that involves at least three breakpoints located on two or more chromosomes. Highly unbalanced gametes may lead to infertility or congenital malformations. Here is reported a double rearrangement considered as the simplest possible CCR and, in a sense, not a true CCR, meiotic segregation for a 46,XY,t(3;6)(p24;p21.2),inv(8)(p11;2q21.2) male patient referred after his partner had undergone three early miscarriages. Sperm fluorescence in-situ hybridization was used to screen for translocation and inversion segregation and an interchromosomal effect (ICE) for 13 chromosomes not involved in CCR. The malsegregation rates for the reciprocal translocation and pericentric inversion were 61.2% and 1.7%, respectively. ICE analysis revealed that the observed chromosome aneuploidy rates of between 0.1% and 0.8% did not differ significantly from control values. A slight increase in cumulative ICE ($P = 0.049$) was observed in the patient, relative to control spermatozoa (with rates of 4.6% and 3.1%). The sperm DNA fragmentation rate differed significantly from control values (5.0%; $P = 0.001$). Reciprocal translocation had no impact on meiotic segregation of the pericentric inversion in this double rearrangement. No conclusion could be drawn regarding the impact of pericentric inversion on translocation.

KEYWORDS: complex chromosome rearrangement, interchromosomal effect, inversion, sperm FISH, translocation

Introduction

A complex chromosome rearrangement (CCR) can be defined as a balanced or unbalanced structural chromosomal aberration that involves at least three breakpoints located on two or more chromosomes, with exchange of genetic material (Pai et al., 1980). These rearrangements can be classified into three groups: (i) three-way exchange CCR, which are generally hereditary and characterized by translocations involving three chromosomes; (ii)

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exceptional CCR, which are mainly *de novo* and characterized by one chromosome with at least two breakpoints; and (iii) double rearrangement CCR defined by at least two independent structural rearrangements and considered as the simplest CCR, but in a sense not a true CCR. The greater the number of breakpoints, the higher the risk of an abnormal phenotype (Pai et al., 1980).

In men, CCR can lead to infertility through the failure of spermatogenesis (Joseph and Thomas, 1982; Rodriguez et al., 1985). Only 12 of 130 patients with CCR are fertile (Goumy et al., 2006; Grasshoff et al., 2003). CCRs and meiotic malsegregation can also result in unbalanced gametes, in which partial duplication/deletion causes recurrent spontaneous miscarriage or (in surviving infants) mental retardation and/or congenital abnormalities.

It was initially reported that the parents of children with Down's syndrome had a greater incidence of translocation (Lindenbaum et al., 1985). Although some sperm fluorescent *in situ* hybridization (FISH) series have confirmed this interchromosomal effect (ICE) (Anton et al., 2010; Blanco et al., 2000; Kirkpatrick et al., 2008; Pellestor et al., 2001), many others have not (Douet-Guilbert et al., 2005; Martin et al., 1990; Schinzel et al., 1992; Warburton, 1985). Hence, there is still much debate as to whether chromosomal rearrangements perturb the meiotic behaviour of chromosomes that are not involved in the abnormality. If it is assumed that this type of ICE exists, one can also legitimately suppose that it is more intense in CCR.

As far as is known, only four studies of meiotic segregation and CCR have been reported: 46,XY,t(5;11)(p13;q23.2), t(7;14)(ql1;q24.1) (Burns et al., 1986) and 46,XY,t(2;11;22)(q13;q23;q11.2) (Cifuentes et al., 1998) using hamster techniques and whole-chromosome painting probes for the second one, a 46,XY,t(1;19;13)(p31;q13.2;q31)mat three-way familial translocation (Loup et al., 2010) and a 46,XY, t(5;13;14)(q23;q21;q31) (Pellestor et al., 2011) using sperm FISH. The malsegregation rates were at 86.3%, 86.5%, 75.9% and 73.0%, respectively.

Here is reported the first meiotic segregation study of a double rearrangement 46,XY,t(3;6)(p24;p21.2),inv(8)(p11;2q21.2) that involved reciprocal translocation and pericentric inversion. The study's objective was to: (i) evaluate the potential impact of inversion or translocation on each other; and (ii) assess the effect of the double rearrangement on ICE by using specific probes for chromosomes 7, 9, 11, 12, 13, 15, 16, 17, 18, 20, 21, X and Y. Sperm DNA fragmentation, which is known to be high in single chromosome rearrangements, was also studied.

Materials and methods

Patient

A non-consanguineous couple was referred for genetic counselling after three spontaneous miscarriages within the first 12 weeks of pregnancy. Karyotyping revealed a 46,XY,t(3;6)(p24;p21.2),inv(8)(p11;2q21.2) double rearrangement (**Figure 1**). The spouse had a normal karyotype. Parental karyotyping was also performed and showed a *de novo* origin for both rearrangements. According to the World Health Organization criteria (WHO, 2010), this

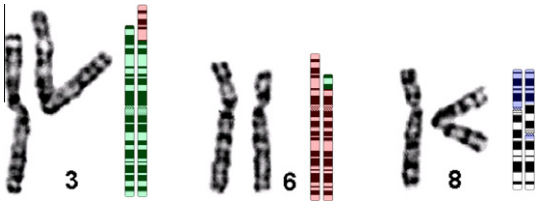

Figure 1 The partial G-banded karyotype of a patient with a balanced double rearrangement 46,XY,t(3;6)(p24;p21.2),inv(8)(p11;2q21.2) and chromosome ideograms.

27-year-old man had normal sperm parameters in terms of the sperm count (425×10^6/ml), progressive motility (40%), vitality (72%) and normal morphology (13%). A control group of three men with normal sperm parameters and karyotypes (consulting for female infertility) was also studied. The control mean (± standard deviation) sperm parameters were as follows: sperm count $191 \pm 21.2 \times 10^6$ per ejaculate; progressive motility 57 ± 13%; percentage with a typical morphology 39 ± 20%; and sperm vitality 85 ± 7%. After genetic counselling, patients provided informed consent for sperm chromosomal evaluation and genetic analysis. This case was part of a broader study of the genetic aspects of infertility that had been approved by the local Institutional Review Board (reference 01032).

Sperm preparation

After seminal liquid elimination, the spermatozoa were washed twice with sterile water (300*g* for 10 min), fixed with Carnoy's solution and then spread on a slide as previously described (Vialard et al., 2008) for sperm FISH and DNA fragmentation assays.

Sperm FISH analysis

Different probe mixtures were used to assess: (i) the reciprocal translocation t(3;6)(p24;p21.2) segregation pattern, using chromosome 3 (red) and 6 (aqua) centromeric probes and the chromosome 6 short-arm telomere probe (green) (Abbott Laboratories, Chicago, IL, USA); (ii) the pericentric inversion inv(8)(p11;2q21.2) segregation pattern, using the chromosome 8 short- (green) and long- (red) arm telomere probes and the chromosome 8 centromeric (aqua) probe (Abbott Laboratories); and (iii) ICE, with five mixtures containing probes for chromosomes not involved in double rearrangement (Abbott Laboratories): (a) centromeric probes for chromosomes 7 (red), 9 (aqua), 15 (green); (b) centromeric probes for chromosomes 11 (red) and 12 (green); (c) specific probes for chromosomes 13 (green) and 21 (red); (d) centromeric probes for chromosomes 16 (green), 17 (aqua) and 20 (red); and (e) centromeric probes for chromosomes X (green), Y (red) and 18 (aqua).

After co-denaturation at 73°C for 4 min, hybridization was carried out overnight at 37°C. Slides were washed, counterstained with 4′,6′-diamidino-2-phenylindole (DAPI) and then analysed as previously reported (Vialard et al., 2008) using a Pathvysion Software Smart Capture FISH

system, version 1.4 (Digital Scientific, Cambridge, UK). Per chromosome, 1000 spermatozoa were analysed. For ICE evaluation, aneuploidy rates were compared with control rates.

TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling

Slides were permeabilized with 0.1% sodium dodecyl sulphate sodium citrate for 15 min. After two washes in phosphate-buffered saline (PBS), slides were incubated with labelling solution (*In situ* Cell Death Detection Kit, fluorescein; Roche Molecular Biochemicals, Rotkreuz, Switzerland) for 2 h at 37°C. Next, slides were washed three times in PBS and analysed after counterstaining with DAPI. Spermatozoa with DNA fragmentation fluoresced blue and green, as previously described (Frainais et al., 2010). A total of 1000 spermatozoa were counted for each patient.

Statistical analysis

Using Statview (SAS Institute, Cary, NC, USA) software, the chi-squared test was used to compare patient and control recombinant and aneuploidy rates and the Wilcoxon test was used to compare sperm DNA fragmentation rates. The threshold for statistical significance was set to $P < 0.05$.

Results

Pericentric inversion inv(8)(p11;2q21.2) segregation

The non-recombinant, balanced product rate (inverted and normal chromosomes) was 98.3% (1033 counted spermatozoa). Hence, only 1.7% of the chromosomes were recombinant and unbalanced (dup8q/del8p and dup8p/del8q) (**Table 1**).

Reciprocal translocation t(3;6)(p24;p21.2) segregation

The alternate segregation rate (leading to normal, balanced chromosomal spermatozoa) was 38.8% (409 of the 1054 analysed spermatozoa) and was the preferred segregation mode. All other spermatozoa were unbalanced (61.2%): adjacent I segregation was the most frequent mode (28.0% of the 1054 analysed spermatozoa), followed by 3:1 segregation (19.3%) and adjacent II segregation (12.3%) (**Table 1**).

Table 1 Meiotic segregation of the pericentric inversion inv(8)(p11;2q21.2) and reciprocal translocation t(3;6)(p24;q21.2) in the male carrier of the double chromosomal rearrangement.

Segregation mode	*Genotype*	*Spot colour*	*No. of sperm analysed*	*Rate (%)*
Pericentric inversion inv(8)(p11;2q21.2)				
Normal or balanced	8 or der8	RAG	1016	98.3
Unbalanced recombinant	dup8p/der8q	AGG	7	0.7
	dup8q/der8p	ARR	10	1.0
Subtotal	—	—	17	1.7
Total	—	—	1033	—
Reciprocal translocation t(3;6)(p24;q21.2)				
Alternate	3,6 or der3, der6	RAG	409	38.8
Adjacent I	3, der6	RA	157	14.9
	6, der3	RAGG	138	13.1
Subtotal	—	—	295	28.0
Adjacent II	3, der3	RRG	69	6.5
	6, der6	AAG	61	5.8
Subtotal	—	—	130	12.3
3:1	3	R	39	3.7
	6, der3, der6	RGGAA	22	2.1
	6	AG	19	1.8
	3, der3, der6	RRAG	24	2.3
	der3	RG	38	3.6
	3, 6, der6	RAAG	28	2.7
	der6	A	15	1.4
	3, 6, der3	RRAGG	18	1.7
Subtotal	—	—	203	19.3
Others	—	—	17	1.6
Total	—	—	1054	—
Total recombinant rate	—	—	2087	61.8

Genotype: dup = duplication, der = derivative chromosome; sport colou: A = aqua; G = green; R = red.

The total recombinant rate of the double rearrangement (i.e. the product of the reciprocal translocation and inversion recombinant rates) was 61.8% (**Table 1**).

The interchromosomal effect

In the ICE analysis, 12,097 and 36,213 spermatozoa were counted for the patient carrier of this double rearrangement and the three controls, respectively. For the patient with double rearrangement, the aneuploidy rates ranged from 0.1% to 0.8% per chromosome and did not differ significantly from control rates (ranging between 0.1% and 0.5%) for any of the chromosomes. The total aneuploidy rate was higher in the patient with double rearrangement than in the controls (4.6% versus 3.1%; $P = 0.049$) and was estimated to be 7.1% if the 20 chromosomes not involved in the double rearrangement were considered.

Sperm DNA fragmentation

The sperm DNA fragmentation rate for the patient carrier of this double rearrangement (1165 analysed spermatozoa) was significantly greater than for the controls (with rates of 15.5% and 5.0 ± 1.1%, respectively; $P = 0.001$) and was above the study laboratory's normal cut-off of 13%.

Discussion

Here is reported what is believed to be the fifth known case of sperm meiotic segregation pattern with the double rearrangement. This 46,XY,t(3;6)(p24;p21.2),inv(8)(p11;2q21.2) rearrangement is, as far as is known, the first such chromosomal abnormality to be observed and involves a translocation and an inversion.

Sperm FISH was used for chromosome-specific identification. Since no *in situ* cross-hybridization with other chromosomes was seen with chromosome 3, 6 and 8 probes, a valid chromosome segregation analysis could be performed.

The recombination rate for the pericentric inversion inv(8)(p11;2q21.2) was 1.7%. For an isolated pericentric inversion, the generation of a significant proportion of unbalanced gametes (>5%) would require a minimum inversion size of 100 Mb and the inversion of at least 50% of the chromosome (Anton et al., 2005; Morel et al., 2007). Hence, the low recombination rate observed in the current study was in agreement with this hypothesis, since the inverted segment was 51 Mb in size and affected 33% of chromosome 8 (which represented 1.7% of the haploid autosomal length). This small inversion with a weak probability of being associated with a high recombination rate did not seem to be associated with a higher malsegregation risk when combined with a reciprocal translocation. These findings suggest that reciprocal translocation had no impact on the meiotic segregation of a pericentric inversion.

For the reciprocal translocation, t(3;6)(p24;p21.2), the recombinant rate was 61.8%. Given that highly variable recombinant rates (between 19% and 90%) are observed for patients who are heterozygous for only one reciprocal translocation, conclusions could not be drawn concerning the impact of the pericentric inversion on reciprocal translocation segregation.

By using chromosome 3 and 6 centromeric probes and the chromosome 6 short-arm telomere probe, this study was unable to distinguish segregation with or without crossing over. After assuming that the various segregation models had the same (random) cross-over frequency, it was decided not to consider only the final product — even though, for example, an alternate product could have resulted in an adjacent I product after crossing over.

The total recombinant rate of this double rearrangement was 61.8%. This rate is lower than the values of 86.5%, 75.9% and 73.0% previously reported for the t(2;11;22)(q13;q23;q11.2), t(1;19;13)(p31;q13.2;q31) and t(5;13;14)(q23;q21;q31) three-way CCR, respectively (Cifuentes et al., 1998; Loup et al., 2010; Pellestor et al., 2011) and the value of 86.3% reported for the 46,XY,t(5;11)(p13;q23.2), t(7;14)(q11;q24.1) (Burns et al., 1986).

This difference could be explained by the type of this double rearrangement. The present case observed two independent chromosome rearrangements, one of which (the inversion) was associated with a very low malsegregation rate. The previously reported cases featured translocations involving three different chromosomes and a double translocation rearrangement, with a high known risk of malsegregation.

For the ICE analysis, 13 of the 20 chromosomes not involved in this double rearrangement (chromosomes 7, 9, 11, 12, 13, 15, 16, 17, 18, 20, 21, X and Y) were studied. Aneuploidy rates ranged between 0.1% and 0.8% and did not differ significantly from control values. In contrast, aneuploidy cumulative rate for the 13 analysed chromosomes was significantly greater for the patient carrier of the double rearrangement, compared with the controls (4.6% versus 3.1%, respectively; $P < 0.05$). However, when considering all the 20 chromosomes not involved in chromosome rearrangement, the estimated total aneuploidy rate was 7.1%, which is similar to previously reported rates where aneuploidy rates in controls ranged from 4.1% to 7.7% (Shi and Martin, 2001). Thus, as in a heterozygous man for only one chromosome rearrangement and with a normal sperm count, there is probably no ICE for this patient.

The DNA fragmentation rate of 15.5% was slightly above the study laboratory's 13% normal cut-off and was significantly ($P = 0.001$) higher than control values (5.0%). This finding suggests that double chromosomal rearrangements increase DNA fragmentation, as previously observed for patients with a solely structural rearrangement and prompt apoptosis in unbalanced spermatozoa (Brugnon et al., 2010; Perrin et al., 2011).

Although reciprocal translocation appears not to have an impact on pericentric inversion segregation, the opposite hypothesis could not be proved true. Further studies of a double rearrangement are required to evaluate the reciprocal impact of chromosome rearrangements. It is clear that, in this particular double rearrangement, chromosome segregation is mainly affected by reciprocal translocation segregation and, in terms of the prognosis, could be considered as a simple translocation.

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