Sperm chromosome analysis in two cases of paracentric inversion

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Objective: To examine sperm meiotic segregation in men with paracentric inversions.
Design: Cases reports, literature review.
Setting: Departments of reproductive biology, cytogenetics, gynaecology, and obstetrics.
Patient(s): Two patients referred for infertility, heterozygous for a paracentric inversion.
Intervention(s): Fluorescence in situ hybridization (FISH) with specific probes and X/Y/18 centromeric probes on 1,000 spermatozoa for the 2 patients and 10 controls.
Main Outcome Measure(s): Sperm aneuploidy frequency.
Result(s): The FISH analysis using the specific probes for the paracentric inversion indicated low disequilibrium (0.4% and 0.5%). The FISH analysis using X/Y/18 centromeric probes indicated aneuploidy frequencies (0.3% and 1.1%), identical to those of control patients with the same sperm parameters.
Conclusion(s): Paracentric inversion seems to be associated with a very low risk of aneuploidy. A larger study is necessary to explore all chromosome inversions. (Fertil Steril® 2006;xx:xxx. ©2006 by American Society for Reproductive Medicine.)

Key Words: Heterozygous paracentric inversion, spermatozoa, FISH, offspring risk, interchromosomal effect

Paracentric inversion (PAI) is a common rearrangement involving two breaks within the same chromosome arm, followed by the reinsertion of the chromosome segment after a 180° rotation. The PAI are one of the most common forms of chromosome polymorphisms found in nature. The incidence of PAI has not been clearly established, but collaborative studies suggest that it ranges from 0.1%–0.5% in the human population (1–3).

The two largest of many reviews on PAI have presented details on 446 cases (4) and 184 cases (5). The PAIs were noted for all chromosomes and the distribution of breakpoints in the p and q arms was found to be random and the incidence was linked to the relative arm lengths of the genome (4). Chromosomes are not equally involved. For example, the 11q arm is involved in the common inv(11)(q21q23), with no abnormality directly attributable to it in the offspring (6) and this is probably the most frequent PAI. The great majority of PAIs are familial (about 90%), and the others de novo.

The mechanism whereby chromosomal imbalance is generated at meiosis in heterozygotes for PAI is linked to the consequence of crossing-over within the inverted segment with acentric and dicentric recombinant formations. Although PAIs in humans are generally harmless (5), recombinant chromosomes have been observed and the risk of viable recombinants has been estimated at 3.8% (4).

In fact, the reproductive risks linked to PAI seem to depend on the size of the inversion and of the chromosomes involved. Stable pseudodicentric chromosomes have been described for chromosomes 9, 14, and 18 (7). Furthermore, an interchromosomal effect related to PAI has been posited, and would explain the birth of children bearing unrelated chromosomal abnormalities (2, 8).

Five studies (only one analyzing more than 500 spermatozoa) on sperm have evaluated the recombinant frequency of PAI (9–13). They have shown a low frequency of recombinant aneuploidy. Interchromosomal effects, which were investigated in only one study (Brown et al. on 282 studied spermatozoa) (10), were not demonstrated.

To complete these findings, we report on two new cases of PAI and their FISH sperm analysis.

CASE REPORTS

Patient 1 was a 43-year-old healthy man referred for two first-trimester miscarriages of his wife, after the birth of one healthy boy and an extrauterine pregnancy. Clinical

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TABLE 1

Sperm characteristics.

Parameters	Concentration (M/mL)	Motility (%)	Morphology (% normal)
Patient 1	108	60	40
Group 1 (n = 5)	90 (45–140)	58 (40–70)	50 (35–60)
Patient 2	30	30	6
Group 2 (n = 5)	40 (25–90)	33 (25–40)	9 (5–14)

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phenotype was normal. The testes were normal in size and consistency. Semen analysis (Table 1) was normal with a 108×10^6/mL concentration, 2.9 mL sperm volume, 60% motility, and 40% normal morphology (David's score). Karyotyping revealed a paracentric inversion of chromosome 11: 46,XY,inv(11)(q13.2;q14.3) (Fig. 1). Analysis of the wife's karyotype, autoimmunity and thrombosis only revealed the presence of anticardiolipin antibodies.

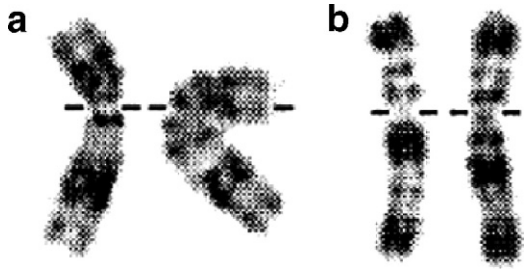
In view of these results, a spermatozoa FISH examination was proposed to evaluate the chromosomal risk for the embryo, studying the segregation of the PAI.

Patient 2 was a 40-year-old healthy man referred for asthenoteratospermia. Semen analysis (Table 1) showed a 30×10^6/mL concentration, 3.5 mL sperm volume, 30% motility, and 6% normal morphology (David's score). In view of the teratospermia, an intracytoplasmic sperm injection (ICSI) procedure was proposed and karyotyping showed a paracentric inversion of chromosome 12: 46,XY,inv(12)(q15q24.1) (Fig. 2). The FISH analysis was then proposed to evaluate the segregation of this inversion.

After being given genetic information and counseling, the patients signed a consent form for chromosomal analysis of sperm, as required by French law.

FIGURE 1

Chromosome 11 of Patient 1 with inv(11)(q13.2; q14.3). The inversion is seen in the right chromosome. (**a**) GTG banding; (**b**) RGH banding.



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MATERIALS AND METHODS

Spermatozoa were fixed in a 3:1 methanol/acetic acid solution after two water washes, spread on a slide, air-dried, and fixed with methanol for 5 minutes at room temperature. Spermatozoa decondensation was performed in 3 N NaOH (1 minute). After dehydration, FISH was performed with a specific telomeric probes mixture (Vysis-Abbott, Abbott Molecular Inc., Des Plaines, IL) to explore the inversion: TelVysion 11p (green) and 11q (red) for patient 1 and TelVysion 12p (green) and 12q (red) for patient 2. We also evaluated the possible interchromosomal effect by analyzing chromosomes X, Y, and 18 with centromeric probes: CenX (green), CenY (red), and Cen18 (aqua) (Vysis-Abbott). The FISH analysis was performed with a 73°C, 4 minutes and 37°C overnight hybridization program.

Slides were washed and counterstained with 6-diamino-2-phenylindole (DAPI) solution and spermatozoa were analyzed with an Olympus microscope using the PathVysion

FIGURE 2

GTG chromosome 12 of Patient 2 with inv(12)(q15q24.1). The inversion is seen in the right chromosome.

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TABLE 2

FISH data for chromosomes X, Y, and 18.

Spermatozoa abnormality	Disomy	Monosomy	Diploidy	Total abnormality	Total spermatozoa analyzed
Patient 1	2	2	0	4	1,221
Control group 1	8	7	1	16	5,129
Patient 2	5	6	0	11	997
Control group 2	49	26	12	87	5,907

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(Digital Scientific, Cambridge, UK). Observation and interpretation criteria were based on the number of spots per probe on the sperm nuclei. For telomeric probes, only spermatozoa with one spot per probe were considered normal, and for chromosomes X, Y, and 18, only spermatozoa with one spot for 18 and one for X or Y were considered normal.

The X/Y/18 results were compared to two patient groups with similar sperm characteristics who had been included in an IVF procedure for tubular infertility (group 1) or abnormal sperm parameters (group 2). Patient 1 was compared with five control patients (group 1) with normal sperm parameters (Table 1) according to World Health Organization (WHO) standards. Mean age was 35 years and 3 months (30 years, 10 months to 39 years, 1 month), sperm concentration 90 (45–140) $\times$ 10^6/mL, motility 58% (40%–70%), and normal morphology 50% (35%–60%). Patient 2 was compared with five control patients (group 2) with abnormal sperm parameters (Table 1). Mean age was 35 years and 10 months (30 years, 12 months to 39 years, 10 months), sperm concentration up to 20 $\times$ 10^6/mL, motility between 20% and 40%, and normal morphology less than 15% (David's score). Sperm concentration was 40 (25–90) $\times$ 10^6/mL, motility 33% (25%–40%), and normal morphology 9% (5%–14%).

Statistical analysis was performed using the Statview (SAS Institute Inc., Cary, NC) program. Differences were considered significant if $p<.05$.

RESULTS

Patient 1

Specific FISH analysis of the paracentric inversion was performed on 1,001 spermatozoa. Only four (0.4%) spermatozoa were diagnosed as abnormal, all with two identical spots. The FISH analysis with X/Y/18 centromeric probes was performed on 1,221 spermatozoa and 3 spermatozoa were abnormal (0.3%), which was statistically identical to the frequency of aneuploidy (0.3%) (16 abnormal spermatozoa over 5,129) in group 1 patients. Frequencies of disomy, monosomy, and diploidy were also similar (Table 2).

Patient 2

Specific FISH analysis of the paracentric inversion was performed on 1,000 spermatozoa. Only five (0.5%) spermatozoa were diagnosed as abnormal, all with two identical spots. The FISH analysis with X/Y/18 centromeric probes was performed on 997 spermatozoa and 11 spermatozoa were abnormal (1.1%), which was statistically identical to the frequency of aneuploidy (1.5%) (87 abnormal spermatozoa over 5,907) in group 2 patients. Disomy, monosomy, and diploidy rate were also similar (Table 2).

DISCUSSION

As previously reported by Martin et al. (9, 11), Brown et al. (10), Devine et al. (12), and Anton et al. (13), we found a very low frequency of PAI recombinant disequilibrium (Table 3): 0.4% for Patient 1 and 0.5% for Patient 2, with more than 1,000 spermatozoa analyzed per patient. These results are comparable to those of Martin et al: 1 of 94 for an inv(7)(q11q22) (9); 0 of 120 for an inv(14)(q24.1q32.1) (11); Devine: 4 of 496 for an inv(2)(q14.2q24.3) (12); and Anton: 24 of 8,158 for an inv(4)(p14p15.3) (13).

We can conclude that PAI is associated with a very low risk of recombination disequilibrium as proposed by Madan and Nieuwint (7). Of course, we have to verify that there is no misdiagnosis between PAI and insertion when there is diagnostic ambiguity, as insertions are sometimes misdiagnosed and paracentric inversions have a lower risk of recombination than insertions. In the 17 cases of recombination reported by Pettenati (4), 15 were probably due to chromosome insertion as suggested by Madan and Nieuwint (7).

We also used FISH analysis of X/Y/18 chromosomes to evaluate the influence of a potential interchromosomal effect. Aneuploidy in Patient 1 was statistically similar to that of our patients with normal sperm parameters (0.4% vs. 0.3%). Aneuploidy was also statistically similar in Patient 2 and our patients with asthenoteratospermia (1.1% vs. 1.5%), and similar to the literature value of 1.5% (14) for patients with abnormal sperm parameters.

We agree with Brown et al. (10) and Martin (11) that there is no interchromosomal effect of PAI and confirm this by

TABLE 3

Results of chromosome segregation in paracentric inversion.

Inversion	Method	No sperm evaluated	% Recombinant sperm	Reference
inv(7)(q11q22),	1	94	0	(9)
inv(9)(q32q34.3)	2	282	0	(10)
inv(14)(q24.1q32.1)	1	120	0	(11)
inv(2)(q14.2q24.3)	2	496	0.81	(12)
inv(4)(p14p15.3)	2	8,158	0.03	(13)
inv(11)(q13.2;q14.3)	2	1,001	0.30	Present study
inv(12)(q15q24.1)	2	1,000	0.50	Present study

Note: 1 = sperm karyotype; 2 = FISH; 3 = sperm typing.

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comparing the results with a control group with the same sperm characteristics.

The recurrent miscarriages of Patient 1's wife were probably due to the presence of anticardiolipin antibodies and not linked to paracentric inversion. As previously reported by Madan (5), PAIs are not usually linked to repeated abortions, for which another etiology is needed. Although recombinant chromosomes may be present in spermatozoa, chromosomally unbalanced offsprings are usually lost very early in pregnancy, perhaps during implantation. Assessment of the recombination risk is of value when discussing prenatal diagnosis, which is often not necessary.

There are no literature reports of a relation between recurrent miscarriages and PAI, or between PAI and altered sperm parameters. The association may be due to chance. The risk of an abnormal karyotype in the offspring can be linked to sperm alteration (15, 16) rather than PAI. Only five patients have been reported and further studies of sperm chromosomes in PAI carriers are required to confirm these results.

Further studies are also needed to elucidate the role of PAI in male factor infertility. Recently, Ichioka et al. (17) have reported a paracentric inversion in the short arm of chromosome 7 associated with nonobstructive azoospermia, suggesting that this inversion might be involved in infertility. A large PAI may induce asynapsis at the pachytene stage and meiotic silencing in unsynapsed chromosomes (MSUC), as described in t(X;16) mice (18). Then, repression may silence some genes necessary for the progression of meiosis. Furthermore, PAI may be confined to the gonads, as suggested by a recent report (19), and an abnormal spermiogram could possibly be related to gonadal mosaicism.

In conclusion, our data confirm that most PAIs can probably be considered as a chromosome variant rather than an abnormality associated with an increased risk. The exceptions are chromosomes 9, 14, or 18, where stable pseudodicentricity has been observed (7). The vast majority of PAIs are likely to be harmless and the risk of an abnormal offspring is expected to be very low.

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