Sperm segregation analysis of a (13;22) Robertsonian translocation carrier by FISH: a comparison of locus-specific probe and whole chromosome painting

T.Anahory1, S.Hamamah1, B.Andréo2, B.Hédon3, M.Claustres4, P.Sarda5 and F.Pellestor2,6

1Service de Médecine et de Biologie de la Reproduction B, Hôpital Arnaud de Villeneuve, 34295 Montpellier Cedex 5, 2CNRS-UPR 1142, Institut de Génétique Humaine, 141 rue de la Cardonille, F-34396 Montpellier Cedex 5, 3Service de Gynécologie Obstétrique, Hôpital Arnaud de Villeneuve, 34295 Montpellier Cedex 5, 4Service de Génétique Moléculaire et Chromosomique, Hôpital Arnaud de Villeneuve, 34295 Montpellier Cedex 5 and 5Service de Génétique Clinique, Hôpital Arnaud de Villeneuve, 34295 Montpellier Cedex 5, France

6To whom correspondence should be addressed. E-mail: Franck.Pellestor@igh.cnrs.fr

BACKGROUND: The t(13;22) Robertsonian translocation constitutes a rare form of rearrangement between acrocentric human chromosomes. Most of the meiotic segregation studies of human Robertsonian translocations have been performed on common t(13;14) and t(14;21) translocations. Analysis of the chromosomal constitution in sperm of Robertsonian translocation carriers is of great interest for assessing the risk of unbalanced forms and adapting genetic counselling. In the present study, we present the first meiotic segregation study of a t(13;22) Robertsonian translocation in human sperm. METHODS: A total of 11 787 sperm nuclei were scored using two distinct FISH labelling techniques, i.e. the locus-specific probes (LSI) method and the whole chromosome painting (WCP) technique. RESULTS: The frequency of normal or balanced sperm resulting from alternate meiotic segregation was 86%. Incidences of unbalanced complements resulting from adjacent segregation modes were 12.79% and 14.36% in LSI and WCP assays, respectively. No significant excess of nullisomy or disomy for the affected chromosomes was observed. CONCLUSIONS: Similar results in segregation were obtained with the two techniques, demonstrating the efficiency of the two strategies for the direct segregation analysis of Robertsonian translocations. The results obtained indicated a moderate meiotic production of imbalance. This study shows that the rare Robertsonian translocation (13;22) displays a similar distribution of balanced and unbalanced sperm patterns as the common Robertsonian translocations previously studied. This suggests that the behaviour of acrocentric chromosomes was similar in all cases of centric fusion.

Key words: FISH/meiotic segregation/Robertsonian translocation/sperm

Introduction

With an incidence of 1.20 per 1000 livebirths, Robertsonian translocation is the most common structural chromosomal aberration found in human (Evans et al., 1978; Nielsen and Wohlert, 1991). The great majority of Robertsonian translocations involve two non-homologous chromosomes and occur between chromosomes 13 and 14 or chromosomes 14 and 21 (73% and 10% of all Robertsonian translocations, respectively) (Therman et al., 1989).

Men carrying Robertsonian translocations have a normal phenotype. However, they may have spermatogenesis alterations expressed by oligozoospermia or azoospermia, and they may be affected by reproductive failure owing to imbalances in chromosome meiotic segregation (Scriven et al., 2001).

The direct analysis of sperm chromosomal constitution can be used to determine meiotic segregation patterns of translocated chromosomes, and to predict the risk of having unbalanced conceptuses. Direct data were first obtained using the human sperm–hamster egg fertilization system. To date, six men heterozygous for Robertsonian translocation have been investigated using this method (Balkan and Martin, 1983; Pellestor et al., 1987; Martin, 1988; Pellestor, 1990; Martin et al., 1992; Syme and Martin, 1992). Although this approach allowed the direct karyotyping of individual human spermatozoa, the procedure remained time-consuming, labour intensive and did not result in the chromosome analysis of large numbers of sperm nuclei (from 24 to 149) (Guttenbach et al., 1997).

Fluorescence in-situ hybridization (FISH) with chromosome-specific DNA probes has offered a new strategy for investigating the meiotic segregation of translocations. Several studies have been carried out, essentially focusing on
the most frequent Robertsonian translocations, i.e. the t(13;14) and t(14;21) (Rousseaux et al., 1995; Escudero et al., 2000; Honda et al., 2000; Morel et al., 2001; Frydman et al., 2001; Anton et al., 2004). Only one case of Robertsonian translocation between two homologous chromosomes, a t(21q:21q), has been investigated by sperm FISH analysis (Acar et al., 2002).

In this report we present the first analysis of sperm chromosome segregation in a man heterozygous for an uncommon (13;22) Robertsonian translocation. Both locus-specific probes and whole chromosome painting probes were used in parallel, in order to compare the efficiency and the accuracy of the two procedures, and to provide complementary data on the male meiotic segregation of this rare chromosomal rearrangement.

Materials and methods

Patient

The patient, aged 38 years, was diagnosed with a (13;22) Robertsonian translocation after 9 years of sexual intercourse without conception associated with oligoasthenozoospermia (concentration 3 x 10^6/ml; 36% normal morphology; 40% progressive motility). Physical examination and sexual development were normal. His wife was healthy with a normal karyotype.

The patient gave his informed consent prior to participation in the study, which was approved by the Ethical Board of the Montpellier Hospital.

Sperm from a fertile, 35-year-old man with normal sperm parameters and a normal karyotype was used as control.

Sperm preparation

Sperm samples were collected in sterile containers after 3 days of sexual abstinence. After liquefying at room temperature, the samples were washed three times in 1 x phosphate-buffered saline by centrifugation (5 min at 2000 rpm). The final pellets were fixed for 1 h in fresh fixative (methanol:glacial acetic acid 3:1) at −20°C. The sperm suspensions were then dropped onto clean microscopic slides and air-dried. Slides were kept for 2 days at room temperature before use in FISH reactions.

Before in-situ labelling reactions, the sperm nuclei decondensation was performed by slide incubation in 25 mmol/l dithiothreitol (Sigma, St Louis, MO, USA), in 1 mol/l Tris–HCl solution at room temperature for 5 min. Slides were then washed twice in 2 x SSC, dehydrated through an ethanol series and air-dried. Slides were kept for 2 days at room temperature before use in FISH reactions.

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FISH procedure

Two distinct probe mixtures were used in this study. The first mixture consisted of Vysis locus-specific probes (LSI), for chromosome 13 (LSI 13, spanning the 13q14 region and labelled in Spectrum-Orange) and chromosome 22 (LSI 22,spanning the 22q13.3 region and labelled in SpectrumGreen). The second probe mixture was composed of two Vysis whole chromosome painting (WCP) probes, i.e. a chromosome 13 painting probe (WCP 13) labelled in SpectrumGreen, and a chromosome 22 painting probe (WCP 22) labelled in SpectrumOrange (Vysis, Downers Grove, IL, USA). The probes were prepared according to the manufacturer’s instructions.

The labelling efficiency of both LSI probes and WCP probes was determined by scoring the proportion of labelled nuclei on samples of 200 metaphase and interphase lymphocytes from the patient and the control subject, in order to assess the efficiency of probe binding and characterisation of the derivative chromosomes. There was no significant difference (P > 0.05) in hybridization efficiency (from 99.60% to 99.88%) between the patient and the control, and the detection of segregation patterns was efficient in both metaphases and interphase nuclei.

Before hybridization, both probe and slide preparations were denatured separately. The probe mixture were denatured for 5 min at 73°C in a water bath, whereas the slides were denatured by immersion in 70% formamide/2 x SSC at 72°C for 3 min, dehydrated again and air-dried.

Each hybridization mixture was applied to the denatured sperm nuclei preparations and slides were covered with coverslips, sealed with rubber cement and hybridized overnight in a dark, moist chamber. Coverslips were then gently removed and the slides were washed for 10 min in a 50% formamide/50% 2 x SSC solution at 46°C, followed by a 10 min wash in 2 x SSC at 46°C, and a 5 min wash in 2 x SSC/0.5% Tween 20 solution, and finally mounted with DAPI (100 ng/ml) in antifade solution.

The slides were examined by two independent observers using a Leitz fluorescence microscope DMRB (Leica SA, Rueil-Malmaison, France), equipped with a DAPI single band-pass, a fluorescein single band-pass filter, a rhodamine single band-pass filter, a fluorescein/rhodamine double band-pass filter, and a triple filter set for simultaneous observation of fluorescein, rhodamine and DAPI signals. Only individual and well-delineated sperm nuclei were scored. Previously described standard assessment criteria were followed for the analysis of in-situ sperm labelling (Pellestor et al., 2001). The scoring criteria were similar for LSI and WCP probes. Briefly, overlapping sperm nuclei, disrupted nuclei or large nuclei with diffuse signals were not considered. Sperm nuclei were scored as having two identical signals when the two spots were of equal size and intensity and were separated by at least the diameter of one hybridization domain. In painting assays, nuclei with two signals of different colours clearly coupled one with the other, were considered as displaying a balanced chromosomal pattern.

Data analysis

The χ²-test was used to statistically analyse the segregation patterns observed in the patient and compare the fluorescent phenotypes between the translocation carrier and the control subject. Comparison of data between the two procedures was also performed using χ²-test. Differences were considered to be significant when P < 0.05.

Results

A total of 11 787 sperm nuclei from the translocation carrier and 10 332 from the control subject were scored. The hybridization efficiencies of the two probe sets reached 99.40% and 99.70% in sperm of patient and donor, respectively.

Among the 11 787 sperm nuclei analysed in the translocation carrier, 4735 nuclei were scored using the WCP probe set (Figure 1) and 7052 nuclei using the LSI probe set (Figure 2). The results are summarized in Table I. In the two assays, similar frequencies of normal and balanced spermatozoa resulting from alternate segregation were found (86.70% and 85.19%, respectively). Unbalanced fluorescent patterns, resulting from adjacent segregation modes, were observed in 12.79% of nuclei scored with LSI probes and 14.36% of nuclei from the WCP assay. There was no...
significant difference \((P > 0.05)\) between these values. In the two assays, the distribution of the different unbalanced patterns (nullisomies and disomies 13 or 22) was similar, with rates of imbalances ranging from 2.66% to 4.24% (Table I).

In control sperm, the proportion of nuclei with normal fluorescent patterns for chromosomes 13 and 22 was 98.9%. Disomies and nullisomies 13 and 22 found in this control sperm ranged from 0.08% to 0.36%. These values were significantly

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**Table I. Segregation of sperm chromosomes for a 13;22 Robertsonian translocation**

<table>
<thead>
<tr>
<th>Segregation types</th>
<th>Chromosomal patterns</th>
<th>In-situ fluorescent phenotypes</th>
<th>FISH results</th>
<th>Proportion (%) of fluorescent phenotypes in control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LSI assay</td>
<td>WCP assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(n)</td>
<td>(%)</td>
</tr>
<tr>
<td>Alternate</td>
<td>13q/22q or der (13q;22q)</td>
<td>Normal</td>
<td>6114</td>
<td>86.70</td>
</tr>
<tr>
<td></td>
<td>13q</td>
<td>Nullisomy 22</td>
<td>222</td>
<td>3.15</td>
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<td></td>
<td>22q/der(13q;22q)</td>
<td>Disomy 22</td>
<td>243</td>
<td>3.44</td>
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<tr>
<td></td>
<td>22q</td>
<td>Nullisomy 3₁</td>
<td>188</td>
<td>2.66</td>
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<tr>
<td></td>
<td>13q/der (13q;22q)</td>
<td>Disomy 13₃</td>
<td>249</td>
<td>3.53</td>
</tr>
<tr>
<td>Adjacent</td>
<td>13q/22q</td>
<td>Normal</td>
<td>4034</td>
<td>85.19</td>
</tr>
<tr>
<td></td>
<td>22q</td>
<td>Nullisomy 22</td>
<td>161</td>
<td>3.40</td>
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<td></td>
<td>22q/der(13q;22q)</td>
<td>Disomy 22</td>
<td>176</td>
<td>3.71</td>
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<tr>
<td></td>
<td>22q</td>
<td>Nullisomy 3₁</td>
<td>142</td>
<td>2.99</td>
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<tr>
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<td>13q/der (13q;22q)</td>
<td>Disomy 13₃</td>
<td>201</td>
<td>4.24</td>
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<tr>
<td>03:00 or diploid</td>
<td>13q/22q/der (13q;22q)</td>
<td>Diploidy</td>
<td>680</td>
<td>14.36</td>
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<tr>
<td></td>
<td>13q/13q/22q/22q</td>
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<td>36</td>
<td>0.51</td>
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<tr>
<td></td>
<td>Diploidy</td>
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<td>21</td>
<td>0.44</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>4735</td>
<td></td>
</tr>
</tbody>
</table>

*In both LSI and WCP assays, the frequency of 13 disomic nuclei exceed the frequency of complementary 13 nullisomies. However, the values don’t display a statistically significant difference \((P > 0.05)\).*

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**Figure 1.** Labelling of sperm nuclei using WCP probes specific for chromosomes 13 and 22 (chromosome 13 in green and chromosome 22 in orange). Various segregation patterns (alternate and adjacent) are observed according to the fluorescent signal distribution.

**Figure 2.** Labelling of sperm nuclei using LSI probes specific for chromosome 13 (LSI 13q14 in orange and LSI 22q13.3 in green). One nucleus displays a normal or balanced fluorescent pattern whereas the other shows an adjacent unbalanced pattern 23,X or 23,Y, −13, + t(13;22).
lower than the rates of imbalances found in the patient. The incidence of double hybridization pattern, corresponding to either diploid sperm nuclei or 3:0 segregation pattern was not significantly different ($P > 0.05$) between the translocation carrier (mean 0.47%) and the control subject (0.37%).

Discussion

This is the first report of the study of a (13;22) Robertsonian translocation. In humans, the (13;22) Robertsonian translocation remains a rare event, with an occurrence estimated at 1% of all Robertsonian translocations (Therman et al., 1989). The few cases reported were ascertainment through trisomy 13 or various population surveys. Thus, in the European collaborative study on prenatal diagnosis, Boué and Gallano (1984) found only three cases of Robertsonian translocation (13;22) among 262 Robertsonian prenatal diagnoses not involving chromosome 21.

To carry out the present FISH study, we utilized two different procedures, i.e. LSI and WCP probes. The LSI method has been used in the majority of previous FISH sperm studies performed in Robertsonian translocation carriers (Rousseaux et al., 1995; Escudero et al., 2000; Honda et al., 2000; Frydman et al., 2001; Acard et al., 2002; Anton et al., 2004), whereas WCPs have been used only once for sperm analysis in three Robertsonian translocation (13;14) carriers (Morel et al., 2001). The use of LSI on sperm can be limited by the DNA compaction and inefficient decondensation of sperm nuclei, or the small size of fluorescent signals usually obtained with these probes. Signals generated by WCP appeared easier to detect in situ on sperm nuclei because of their size and intensity. Previous uses of painting probes on human sperm have demonstrated that this type of probe possessed both the specificity and the sensitivity required for the in-situ scoring of chromosomal imbalances in human sperm (Rives et al., 1998; 1999; Morel et al., 2001). In addition, the use of WCP requires only a moderate sperm decondensation to provide a high hybridization efficiency (Rives et al., 1998).

The combined use of these two labelling procedures allowed us to compare the efficiency of the two techniques on human sperm, and provided an internal control for the analysis of meiotic segregation. Both procedures gave similar results in terms of balanced and unbalanced nuclei. This indicates that FISH with WCP is an accurate approach for sperm study in Robertsonian translocation carriers, and so can be used to complement the LSI technique. Morel et al. (2001) reported that the WCP method allowed the in-situ differentiation of both normal spermatozoa (with two signals of different colour separated by at least one diameter) and balanced spermatozoa (with two signals of different colour coupled one with the other). In our study, although such a distinction was possible in numerous sperm nuclei, a significant proportion of nuclei also displayed partially overlapping signals. This observation lead us to consider that the WCP approach was not efficient enough to precisely estimate the proportion of normal and balanced nuclei resulting from the alternate segregation mode. Consequently, no distinct classification was made between normal and balanced sperm nuclei.

The observed high frequency (89%) of sperm nuclei resulting from alternate segregation is in good agreement with results from previous analysis of sperm in Robertsonian translocation carriers, using either the human–hamster fertilization system or FISH procedure (Pellestor, 1990; Honda et al., 2000; Anton et al., 2004). Similar high incidences of alternate meiotic segregation in sperm of Robertsonian translocation carriers were also observed in other mammalian species such as mice and bulls (Gropp and Winking, 1981; Tateno et al., 1994). All these data support the existence of a similar meiotic behaviour of rearranged chromosomes in Robertsonian translocations. The predominance of alternate segregation over other segregation types does not appear to be influenced by the difference in chromosomes involved in the translocation. Meiotic analyses of trivalent synaptonemal complexes in Robertsonian translocations have shown the predominant pairing of the acrocentric elements in cis-configuration. Such configuration favours alternate meiotic segregation (Vidal et al., 1982; Luciani et al., 1984; Navarro et al., 1991).

The overall frequency of unbalanced spermatozoa resulting from adjacent segregation modes is 12%. This is also consistent with the results of previous FISH studies (results ranging from 10.8% to 22.6%). The similarity of meiotic configurations in Robertsonian translocation could explain the relatively homogeneous rates of imbalances. In addition, both the number and the location of chiasmata could contribute to produce similar proportions of imbalances in all Robertsonian translocations. Because of the correlation between the line of chromosomal segregation and the chiasma line, there is a strong prevalence of alternate segregation, resulting in a low rate of unbalanced spermatozoa, and consequently in a low risk of imbalance in progeny of male carriers. However, the incidences of imbalances in sperm of Robertsonian translocation carriers are always higher than the incidences of imbalances drawn from studies of fetuses (Boué and Gallano, 1984) or newborns (Daniel et al., 1989). This finding indicates that there is a strong in-utero selection against unbalanced conceptuses. In this way, it is interesting to note that studies of female carriers of Robertsonian translocation performed by polar body FISH analysis (Munne et al., 2000; Durban et al., 2001) reported significantly higher rates of unbalanced oocytes (32–36%). Such variations emphasize the difference in the outcome of adjacent segregation in male and female meiosis, probably linked to the weakness of the female meiosis checkpoint mechanisms (LeMaire-Adkins et al., 1997). Also, some reports of preimplantation genetic diagnosis for Robertsonian translocation carriers have indicated elevated rates of imbalanced chromosomal constitution (Conn et al., 1998; Iwarsson et al., 2000; Alves et al., 2002). The observed abnormalities were essentially mosaicism and chaotic chromosomal constitution. However, these data are still in discussion, since other PGD reports with no evidence for high frequency of unbalanced embryo do not support the contention that Robertsonian translocations could predispose to malsegregation, and suggest that the observed chromosomal abnormalities could essentially result from
culture conditions (Scriven et al., 2001). Post-zygotic events affecting the chromosomal segregation during early cleavage stages could also influence the occurrence of unbalanced concepts, and this could be a patient-related phenomenon.

The production of unbalanced gametes renders difficult the investigation of meiotic segregation in Robertsonian translocation based on data from live birth or prenatal diagnoses. The sperm analysis of Robertsonian translocation carriers constitutes a unique and efficient approach for predicting the meiotic behaviour of these chromosomal rearrangements and estimating the risk of occurrence of unbalanced embryo in translocation carrier couples. The present study has shown that the rare Robertsonian translocation (13;22) displayed a similar distribution of balanced and unbalanced sperm patterns as the common Robertsonian translocations previously studied, thus suggesting that the behaviour of acrocentric chromosomes was similar in all cases of centric fusion. Further investigations of other rare Robertsonian translocations are now required to confirm these data.

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References


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