Meiotic segregation in spermatozoa of a 45,XY,-14,der(18)t(14;18)(q11;p11.3) translocation carrier: A Case Report

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A 35-year-old male was found to have a 45,XY,-14,der(18)t(14;18)(q11;p11.3) karyotype during the investigations for a couple with infertility for 8 years. Two sperm samples were obtained and analysed in triple fluorescence in situ hybridization (FISH) with the D18Z1 and LSI IGH/BCL2 probes. The frequency of gametes exhibiting a normal or balanced chromosomal equipment was 87.26 and 90.97% in samples 1 and 2, respectively. No statistically significant difference was found between the results of meiotic segregation of both samples. These proportions are close to those observed among Robertsonian translocation carriers. They can probably be explained by the formation of trivalent in cis configuration during meiosis I between the derivative chromosome and the normal chromosomes 14 and 18, as in Robertsonian translocation carriers. These results suggest that the configuration adopted at pachytene strongly determines the segregation mode that will be preferentially followed during anaphase I.

Key words: meiotic segregation/intra-individual variation/fluorescence in situ hybridization/chromosomal translocation

Introduction

Balanced reciprocal and Robertsonian translocations are the most common structural chromosomal rearrangements in infertile men (De Braekeleer and Dao, 1991; De Braekeleer et al., 2006). Balanced reciprocal translocations result from the exchange of chromosomal material between two heterologous chromosomes without loss or gain of material, giving a 46-chromosome karyotype. Robertsonian translocations involve the fusion of the long arms of two acrocentric chromosomes (13, 14, 15, 21 and 22 pairs), with subsequent loss of their short arms, leading to a 45-chromosome karyotype. Generally, carriers of these balanced reciprocal and Robertsonian translocations have no phenotypic consequences, but they can produce a significant percentage of chromosomally unbalanced gametes (ranging from ~5 to >80%) (Morel et al., 2004a, 2006; Benet et al., 2005; Roux et al., 2005; Midro et al., 2006; Moradkhani et al., 2006; Ogur et al., 2006).

In this study, we analysed and compared the meiotic segregation in spermatozoa from two different samples of a 45,XY,-14,der(18)t(14;18)(q11;p11.3) carrier using fluorescence in situ hybridization (FISH). The case reported here is neither a reciprocal translocation nor a Robertsonian translocation. To our knowledge, this is the first meiotic segregation study in a male carrier of such an abnormality.

Materials and methods

Patient’s history and cytogenetic analysis

A couple (29-year-old female and 35-year-old male) presented with an 8-year history of primary infertility. The female partner had had one miscarriage, but no cytogenetic study was performed on the pregnancy loss. The male partner was found to have a very severe oligoasthenozoospermia on two consecutive semen analyses (0.01 and 0.02 million spermatozoa/ml).

Subsequent karyotyping of the male peripheral blood using G and R banding showed a translocation involving chromosomes 14 and 18 (Figure 1). His karyotype was 45,XY,-14,der(18)t(14;18)(q11:p11.3). A complementary FISH analysis using CEP14/22 (D14Z1 and D22Z1, spectrum green, Cytocell, Compiègne, France) and 18p subtelomere-specific probe (D18S552, spectrum red, Cytocell) allowed the accurate identification of the translocation. The karyotype was rewritten as 45,XY,-14,der(18)t(14;18)(q11;p11.3)ish der(18)(q11;p11.3)(D18S552+,D14Z1–,D22Z1–).
The results of a physical examination were normal, and there was no evidence of mental retardation. Familial cytogenetic investigations showed the patient’s parents to have a normal karyotype.

Sperm analyses
Sample collection
Before this study, the patient was informed of the investigations and gave his consent. Two sperm samples were obtained at >3 months interval. Given the low sperm concentration, the spermogram and the spermocytogram were not performed again, the whole sample being used for FISH analysis.

Analysis of the meiotic segregation
Detailed procedures for sperm preparation and FISH have been previously described (Morel et al., 2004c; Douet-Guilbert et al., 2005). Triple FISH was carried out using the specific alphoid probe of chromosome 18 (D18Z1, spectrum aqua, Abbott, Rungis, France) and LSI IGH/BCL2 probes which are a mixture of the LSI IGH probe (~1.5 Mb, spectrum green, Abbott) and the LSI BCL2 probe (~750 kb, spectrum orange, Abbott).

Ideogram showing the localization of the DNA probes and the various segregation patterns after analysis using a Zeiss Axioplan microscope (Zeiss, Le Pecq, France) and Isis (significant in situ imaging system) (MetaSystems, Altussheim, Germany) are schematized in Figure 2. Sperm nuclei were analysed using strict selection criteria (Morel et al., 1997). All spermatozoa presenting at least one green and/or orange and/or aqua signal were scored.

Statistical analysis was carried out using the chi-square with correction for small numbers. The significance level was set at $P < 0.05$.

Results
A total of 981 (sample 1) and 144 (sample 2) spermatozoa were analysed after triple FISH. The results of the meiotic segregation in both samples are summarized in Table I. For samples 1 and 2, respectively, 87.26 and 90.97% of the analysed nuclei showed one orange, one green and one blue signal corresponding to a normal or balanced chromosomal complement resulting from alternate segregation.

The proportion of chromosomally unbalanced spermatozoa resulting from the adjacent mode was estimated at 9.08 and 6.95% (samples 1 and 2, respectively). No statistically significant difference was found in the proportion of nuclei showing two orange, one green and two blue signals and those with one
green signal, or between the rate of spermatozoa showing one orange, two green and one blue signal and those with one orange and one blue signal.

No statistically significant difference was found between the results of meiotic segregation of both samples \((P > 0.05)\).

**Discussion**

In this study, we report the results of the meiotic segregation in two different samples from a 45,XY,-14,der(18)t(14;18)(q11:p11.3) carrier. The patient has a normal phenotype because this translocation results in the formation of a metacentric chromosome composed of the whole chromosome 18 (18p subtelomere present) and the long arm of chromosome 14. The loss of the short arm of chromosome 14 is without consequence because it only contains nucleolar organizer genes. However, as a consequence of this rearrangement, the patient has only 45 chromosomes.

Meiotic segregation studies in males carrying a reciprocal translocation showed the proportion of chromosomally unbalanced spermatozoa to vary from 10 to >80% (Morel et al., 2004a, 2006; Benet et al., 2005). In males carrying a Robertsonian translocation, meiotic segregation studies showed most spermatozoa to be chromosomally normal or balanced (ranging from 72 to 93%) (Morel et al., 2004a; Roux et al., 2005; Brugnon et al., 2006; Ogur et al., 2006).

The frequency of gametes exhibiting a normal or balanced chromosomal equipment in the patient reported here was 87.26 and 90.97% in samples 1 and 2, respectively. These proportions are close to those observed among Robertsonian translocation carriers. In Robertsonian translocation carriers, during prophase I meiosis, pairing of the involved chromosomes gives a trivalent structure (Vidal et al., 1982; Luciani et al., 1984). Thus, the results of meiotic segregation in spermatozoa of this 45,XY,-14,der(18)t(14;18)(q11:p11.3) carrier can probably be explained by the formation of trivalent in cis configuration during meiosis I between the derivative chromosome and the normal chromosomes 14 and 18, as in Robertsonian translocation carriers.

Theoretically, in Robertsonian translocation carriers and in the patient reported here, cis or trans configurations are possible (Figure 2). Luciani et al. (1984) using meiotic cytogenetics on testicular biopsies found that the trivalent was always in cis configuration (Luciani et al., 1984). Moreover, it is well known that the cis configuration tends to segregate in an alternate way, producing chromosomally balanced and normal spermatozoa. However, a small proportion of unbalanced gametes deriving from adjacent or 3:0 segregation is also produced. Moreover, Luciani et al. (1984) found an association of trivalent and sex vesicle in most of the nuclei at the pachytene stage in a sterile t(13;14) carrier (Luciani et al., 1984). This association could lead to gametogenic arrest and produce severe spermatogenetic impairment (Johannisson et al., 1993; Gabriel-Robeze and Rumpler, 1996).

The results of meiotic segregation in both samples showed no variations. To the best of our knowledge, a single study on intra-individual variations was previously published; no difference was found in the meiotic segregation profiles between two samples from a male carrier of a t(9;22)(q21;q11.2) (Morel et al., 2004b). Four studies have analysed the meiotic segregation of translocations within families; similar profiles were found in each family but not between families (Estop et al., 1992; Rousseaux et al., 1995; Cora et al., 2002; Morel et al., 2004c, 2006).

As the frequency of normal or balanced gametes was ~90%, it was decided to try an IVF with microinjection. Ten oocytes were collected, 3 fertilized, but a single embryo was obtained and transferred, without pregnancy. A new attempt will be made during the year.

In conclusion, this study shows that the configuration adopted at pachytene strongly determines the segregation mode that will be preferentially followed during anaphase I.

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**References**


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**Table I. Results of the meiotic segregation in two different samples from a 45,XY,-14,der(18)t(14;18)(q11:p11.3) carrier**

<table>
<thead>
<tr>
<th>Fluorescence signals</th>
<th>Segregation modes</th>
<th>Chromosomal equipment</th>
<th>Number (%) of sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGB</td>
<td>Alternate</td>
<td>14/18 or der(18)t(14;18)</td>
<td>856 (87.26)</td>
</tr>
<tr>
<td>OOGGBB</td>
<td>Adjacent</td>
<td>18/der(18)t(14;18)</td>
<td>9 (0.92)</td>
</tr>
<tr>
<td>G</td>
<td>Adjacent</td>
<td>14</td>
<td>4 (0.41)</td>
</tr>
<tr>
<td>OGGB</td>
<td>Adjacent</td>
<td>14/der(18)t(14;18)</td>
<td>46 (4.69)</td>
</tr>
<tr>
<td>OB</td>
<td>Adjacent</td>
<td>18</td>
<td>30 (3.06)</td>
</tr>
<tr>
<td>OOGGBB</td>
<td>3:0 or diploidy</td>
<td>14/18/der(18)t(14;18)</td>
<td>18 (1.83)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td>18 (1.83)</td>
</tr>
</tbody>
</table>

B. blue; G, green; O, orange.


