Segregation of chromosomes in sperm of a t(X;18)(q11;p11.1) carrier inherited from his mother: Case Report

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Balanced reciprocal translocations are the most common structural abnormalities; most involve two autosomes while a few involve a gonosome (X or Y chromosome) and an autosome. These rearrangements are usually associated with infertility and/or a higher risk of chromosomal imbalances among offspring. This 26 years old man was first seen because of a 3-year history of primary infertility. He had been found to have a translocation, t(X;18)(q11;p11.1), inherited from his mother when he was 9 years old. Semen analysis showed a very severe oligoasthenoteratozoospermia (OAT). A total of 447 spermatozoa were analysed using three-colour fluorescent in situ hybridization (FISH). The alternate segregation pattern, leading to a normal or balanced chromosomal content, was found in 54.36% of the spermatozoa studied. The frequencies of Adjacent I, Adjacent II, 3:1 segregation and diploidy (or 4:0 segregation) were 8.28, 5.14, 22.37 and 2.01%, respectively. Balanced reciprocal translocations between an autosome and the X chromosome lead to important disruptions in human spermatogenesis. Almost all the males with an X-autosome translocation have azoospermia. The man reported here had very severe OAT and is the first in whom the meiotic segregation pattern was analysed. This case further emphasizes the interest in performing FISH studies in infertile males with a chromosomal translocation to provide them with a personalized imbalance risk.

Keywords: meiotic segregation; FISH; X-autosome translocation

Introduction

Balanced reciprocal translocations are the most frequent structural chromosomal abnormalities in humans. Their frequency is estimated at 0.12% in the general population but is 6.5 times more frequent among infertile males (De Braekeleer and Dao, 1991; De Braekeleer et al., 2006). They result from material exchange between two non-homologous chromosomes, without loss or gain of material. The majority of reciprocal translocations occurs between two autosomes. Translocations involving gonosomes are rare and belong to three subgroups: Y-autosome translocations (Hsu, 1994; Giltay et al., 1997; Brisset et al., 2005; Pinho et al., 2005), X-autosome translocations (Fraccaro et al., 1977; Lee et al., 2003; Ma et al., 2003; Ishikawa et al., 2007) and X–Y translocations (Yamada et al., 1982; Gabriel-Robez et al., 1990; Taiar et al., 1995; Morel et al., 2001). Most of the men carrying a translocation involving gonosomes have azoospermia (Lee et al., 2003; Brisset et al., 2005; Ishikawa et al., 2007), although some show a severe oligozoospermia (Fraccaro et al. 1977; Giltay et al. 1997; Morel et al. 2001; Ma et al., 2003).

Meiotic segregation, studied by heterospecific fecundation or fluorescent in situ hybridization (FISH), of >80 men carrying a translocation between two autosomes has been reported (Morel et al., 2006). However, there are only two reports on males having a Y-autosome translocation (Mennicke et al., 1997; Giltay et al., 1999) and a sole report concerning a t(X;Y) (Morel et al., 2001). To our knowledge, we report here the first meiotic segregation analysis in a male carrying an X-autosome translocation.

Case Report

Patient

The patient was first seen when he was 9 years old, because of his parents’ secondary infertility following his birth. The cytogenetic analysis revealed a 46,Y,t(X;18)(q11;p11.1) karyotype, inherited from his mother.

The couple (female 24 years old; male 26 years old) presented with a 3-year history of primary infertility. The female partner had a normal karyotype. Semen analysis
showed a very severe oligoasthenoteratozoospermia with 0.4 million spermatozoa/ml, 95% immobile sperm and 90% abnormal forms. Prior to this study, the patient was informed of the investigations and subsequently gave his consent.

**Meiotic segregation analysis**

Triple FISH was carried out using the specific alphoid probe of chromosome 18 (D18Z1, spectrum aqua, Abbott, Rungis, France), the 18p subtelomere probe (tel 18p, spectrum green, Abbott) and the subtelomere Xq/Yq probe (telXq/Yq, spectrum orange, Abbott). An ideogram showing the translocation, the localization of the probes and the quadrivalent is shown in Fig. 1.

The sperm sample of the patient was also analysed in triple FISH with specific alphoid probes of chromosomes Y (DYZ3, spectrum orange, Abbott) and 18 (D18Z1, spectrum aqua, Abbott) and the 18p subtelomere probe (tel 18p, spectrum green, Abbott).

Detailed procedures for sperm preparation and FISH have been previously described (Morel et al., 2004b; Douet-Guilbert et al., 2005; Morel et al., 2007). The slides were analysed using a Zeiss Axio Plan microscope (Zeiss, Le Pecq, France). Subsequent image acquisition was performed using a CCD camera with Isis (significant in situ imaging system) (MetaSystems, Altlesuheim, Germany).

Spermatozoa with one green, one orange and one blue signal were classified as normal or balanced whereas spermatozoa with other signal combinations were scored as unbalanced.

**Results**

A total of 447 spermatozoa was analysed (Table I). A preferential alternate segregation mode was observed with a rate of 54.36%. The probe combination could not distinguish whether the studied spermatozoa had a chromosomally balanced or normal content.

All other spermatozoa were unbalanced (45.64%). Among the unbalanced spermatozoa, the 3:1 segregation mode was the most frequent with 22.37% of analysed spermatozoa, followed by the Adjacent I (8.28%) and Adjacent II modes (5.14%). Moreover, 2.01% of the spermatozoa were diploid or segregated in the 4:0 mode. The remaining spermatozoa (7.83%) showed ambiguous signals or hybridization failure (Caer et al., 2007).

A total of 287 spermatozoa was analysed in triple FISH using DYZ3, D18Z1 and tel18p probes (Table II). The frequency of spermatozoa exhibiting one orange spot, one blue spot and one green spot was assessed at 29.27%. We could not distinguish normal (23,Y) from unbalanced (24,Y,der(X)) spermatozoa as they showed the same fluorescent combination. Nevertheless, with triple FISH using D18Z1, tel18p and telXqYq, we had observed that 2.68% of spermatozoa were 24,Y,der(X) (Table I). Thus, using this correction factor, we estimated that 26.59% (29.27–2.68) was the rate of normal spermatozoa (23,Y).

**Discussion**

Balanced reciprocal translocations between two autosomes are the most common structural chromosomal rearrangements in humans. X-autosome translocations are rare and usually of maternal origin or arising de novo (Kalz-Fuller et al., 1999). In general, female carriers exhibit normal reproductive function (Cantu et al., 1985; Ma et al., 2003; Panasiuk et al., 2004) while

![Figure 1: Ideogram showing the t(X;18)(q11;p11.1), the localization of the probes and the quadrivalent at the pachytene stage](image)

**Table I.** Results of the meiotic segregation in the sample from the 46,Y,t(X;18)(q11;p11.1) carrier using triple FISH with D18Z1, tel18p and telXq/Yq probes.

<table>
<thead>
<tr>
<th>Fluorescent signals</th>
<th>Segregation modes</th>
<th>Chromosomal content</th>
<th>n (%) by combinations</th>
<th>% by mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGB</td>
<td>Alternate</td>
<td>Y/18 der(X)/der(18)</td>
<td>243 (54.36)</td>
<td>54.36</td>
</tr>
<tr>
<td>GGB</td>
<td>Adjacent I</td>
<td>der(X)/18 Y</td>
<td>16 (3.58)</td>
<td>8.28</td>
</tr>
<tr>
<td>OOB</td>
<td></td>
<td>Y/der(18)</td>
<td>21 (4.70)</td>
<td></td>
</tr>
<tr>
<td>OG</td>
<td>Adjacent II</td>
<td>der(X)/Y 18/der(18)</td>
<td>12 (2.68)</td>
<td></td>
</tr>
<tr>
<td>OGBB</td>
<td>Adjacent II + crossing-over</td>
<td></td>
<td>9 (2.01)</td>
<td></td>
</tr>
<tr>
<td>OOBBB</td>
<td></td>
<td></td>
<td>2 (0.45)</td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td>3:1</td>
<td>18 der(X)/Y/der(18)</td>
<td>34 (7.61)</td>
<td>22.37</td>
</tr>
<tr>
<td>OOGGB</td>
<td></td>
<td>der(18)/Y/der(18)</td>
<td>12 (2.68)</td>
<td></td>
</tr>
<tr>
<td>OB</td>
<td></td>
<td>der(18)/Y/18 der(X)</td>
<td>12 (2.68)</td>
<td></td>
</tr>
<tr>
<td>OGGGB</td>
<td></td>
<td>Y/der(18)/18 der(X)</td>
<td>1 (0.23)</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td></td>
<td>Y/der(18)/18 der(X)</td>
<td>9 (2.01)</td>
<td>2.01</td>
</tr>
<tr>
<td>OOGGBB</td>
<td></td>
<td>der(X)/der(18)/18</td>
<td>35 (7.83)</td>
<td>7.83</td>
</tr>
</tbody>
</table>

*O, orange; G, green; B, blue.
*Ambiguous signals or hybridization failure.
male carriers have azoospermia (Quack et al., 1988; Solari et al., 2001; Lee et al., 2003; Ishikawa et al., 2007).

Histologic examinations of biopsied testicular tissue from males carrying a X-autosome translocation show spermatogenic arrest at the primary spermatocyte level with most of the cells being arrested at the pachytene stage (Quack et al., 1988), although a few germ cells at the spermatid level are sometimes found (Ishikawa et al., 2007). Indeed, balanced reciprocal translocations between an autosome and the X chromosome lead to important disruptions in human spermatogenesis. This is explained by the fact that the derivative X chromosome may be interfering in an abnormal sexual vesicle formation, leading to meiotic disturbances and, consequently, to spermatogenetic arrest.

Two males carrying a X-autosome translocation and a severe oligozoospermia have been reported thus far, one carrying a t(X;15)(p11.3:p1) (Fraccaro et al., 1977), the other, a t(X;20)(q10;q10) (Ma et al., 2003). In this latter case, the translocation was inherited from his mother and transmitted to his daughter through ICSI (Ma et al., 2003). Two other males have each fathered a child, one having a t(X;14)(p−;q+) (Buckton et al., 1971), the other, a t(X;1)(p−;q+) (Leichtman et al., 1978).

Mattei et al. (1982) studied the cytogenetic characteristics of the X-autosome translocations. They found that the breakpoints distribution on the X chromosome did not differ significantly from the expected distribution (Mattei et al., 1982). Interestingly, in two subjects each with a balanced t(X;autosome), one of whom had one 46,XY normal son (although paternity was not proven) (Buckton et al., 1971; Leichtman et al., 1978), and in three t(X;autosome) carriers with severe oligozoospermia (Fraccaro et al., 1977; Ma et al., 2003) (our case), the breakpoint on the X chromosome appeared to be always in the pericentromeric region or at the centromere. These balanced translocations may have fewer deleterious effects on spermatogenesis than other X chromosome breakpoints. Thus, when the breakpoint is located near the centromere of the X chromosome, a few germinal cells could be able to complete the meiotic process by producing spermatozoa.

Table II. Results of the meiotic segregation in the sample from the 46,Y; t(X;18)(q11;p11.1) carrier using triple FISH with DYZ3, D18Z1 and tel18p probes.

<table>
<thead>
<tr>
<th>Fluorescent signals</th>
<th>Chromosomal content</th>
<th>n (%) by combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGB</td>
<td>Y/18</td>
<td>84 (29.27)</td>
</tr>
<tr>
<td></td>
<td>der(X)/Y/18</td>
<td>18 (6.75)</td>
</tr>
<tr>
<td></td>
<td>der(X)/der(18)</td>
<td>91 (34.15)</td>
</tr>
<tr>
<td></td>
<td>der(18)</td>
<td>19 (7.10)</td>
</tr>
<tr>
<td>BG</td>
<td>der(X)/Y/18</td>
<td>32 (11.15)</td>
</tr>
<tr>
<td></td>
<td>Y/der(18)</td>
<td>15 (5.60)</td>
</tr>
<tr>
<td></td>
<td>der(X)/Y/der(18)</td>
<td>8 (2.90)</td>
</tr>
<tr>
<td></td>
<td>der(X)/Y</td>
<td>14 (5.20)</td>
</tr>
<tr>
<td>BO</td>
<td>18/der(18)</td>
<td>22 (7.67)</td>
</tr>
<tr>
<td></td>
<td>der(X)/Y/der(18)/18</td>
<td>8 (2.79)</td>
</tr>
<tr>
<td></td>
<td>der(X)/Y/der(18)/18</td>
<td>27 (9.41)</td>
</tr>
<tr>
<td>BBG</td>
<td>18/der(18)</td>
<td>2 (0.70)</td>
</tr>
<tr>
<td>BBGO</td>
<td>18/der(18)/18 diploidy</td>
<td>8 (2.79)</td>
</tr>
<tr>
<td>Others*</td>
<td></td>
<td>27 (9.41)</td>
</tr>
</tbody>
</table>

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

*Ambiguous signals or hybridization failures.

To our knowledge, this is the first cytogenetic study of the chromosomal content in the spermatozoa of a man with a X-autosome translocation. Solely, a small number of spermatozoa could be analysed because of his severe oligozoospermia. The majority of the analysed nuclei showed normal or balanced equipment resulting from alternate segregation with normal (23,Y) or balanced (23,der(X),der(18)) spermatozoa in similar proportions. A 23,Y or a 23,der(X),der(18) spermatozoon fertilizing a normal 23,X oocyte will lead, respectively, to the birth of a 46,XY boy or a 46,X,t(X;18)(q11;p11.1) girl carrying the same translocation as her father and her grandmother. Nevertheless, balanced t(X-autosome) in girls is not necessarily associated with the same phenotype as that of the mother or, as in this case, as that of the grandmother. As proposed by Ma et al. (2003), it is of clinical significance to determine to what degree skewed X chromosome inactivation is present in the resulting female newborns with X-autosome translocation so that the parents can be counselled accordingly (Ma et al., 2003).

The frequency of gametes exhibiting a chromosomal unbalanced equipment was 45.64% with the preferential mode of imbalance being the 3:1 mode. This preferential unbalanced 3:1 segregation is particularly described in the t(11;22)(q23;q11) (Estop et al., 1999; Geneix et al., 2002; Escudero et al., 2003). The same results were found in other reciprocal translocations involving small or acrocentric chromosomes (Geneix et al., 2002; Escudero et al., 2003) or with breakpoints close to the telomeric regions (Martini et al., 1998; Escudero et al., 2003). The 3:1 mode is also favoured when chromosome 9 is involved in the translocation or when, as in our patient, breakpoints are near the centromere (Simpson and Bischoff, 2002; Trappe et al., 2002; Rives et al., 2003; Brugnon et al., 2006).

Theoretically, in the 3:1 mode, gametes with 22 or 24 chromosomes should be found in equal proportions. However, in our case, 15.22% of the spermatozoa had 22 chromosomes and 7.15% had 24 chromosomes. These differences could be explained by a differential viability of the spermatocytes and/or spermatids according to their chromosomal content (Estop et al., 1992; Blanco et al., 1998), but more likely, by the overestimation of the frequency of spermatozoa with 22 chromosomes, possibly due to technical problems (Morel et al., 2004).

Meiotic segregation patterns of men carrying a balanced reciprocal translocation between two autosomes have been recently reviewed (Morel et al., 2006). The frequency of unbalanced spermatozoa was shown to vary from 19% to >80%. Overall, the frequencies of normal/balanced and unbalanced gametes were, on average, 44% and 56%, respectively. Thus, the risk for X-autosome translocation carriers of producing a chromosomally unbalanced offspring is not higher than that for carriers of a reciprocal translocation between two autosomes, at least in our patient.

During genetic counselling, preimplantation genetic diagnosis (PGD) and conventional prenatal diagnosis were discussed with the couple. As the waiting list for PGD is very long in France, given the frequency of normal or balanced gametes of ~55%, the couple decided to try an in vitro fertilization.
with sperm microinjection followed by conventional prenatal diagnosis. There were 19 oocytes collected, 12 injected and 6 embryos obtained. One embryo was transferred and a 46,XY newborn baby with no malformation was born.

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References


