Meiotic studies in two human reciprocal translocations and their association with spermatogenic failure

M.Oliver-Bonet1,2, J.Benet3, F.Sun1,2, J.Navarro3, C.Abad4, T.Liehr5, H.Starke5, C.Greene6, E.Ko2 and R.H.Martin1,2,7

1University of Calgary, 6Department of Obstetrics and Gynecology, Faculty of Medicine, University of Calgary, Calgary T2N 4N1, 2Department of Genetics, Alberta Children’s Hospital, Calgary T2T 5C7, Canada, 3Unitat de Biologia, Facultat de Medicina, Departament de Biologia Cellular, Fisiologia i d’Immunologia, Universitat Autònoma de Barcelona, Bellaterra 08193, 4Corporació Sanitària Parc Taulí, 08208, Sabadell, Spain and 5Institute of Human Genetics and Anthropology, D-07740 Jena, Germany

7To whom correspondence should be addressed at: Department of Genetics, Alberta Children’s Hospital,1820 Richmond Road S.W., Calgary, Alberta, Canada T2T 5C7. E-mail: rhmartin@ucalgary.ca

BACKGROUND: Reciprocal translocations are often associated with infertility in male carriers. However, some carriers present normal semen profiles and are identified because of repetitive pregnancy failures. METHODS: Here, we report two different cases of reciprocal translocations. The first patient carried a t(10;14) and was normozoospermic. The second patient carried a t(13;20) and was azoospermic. Synaptonemal complexes from both carriers were analysed using immunocytogenetic techniques and multi-centromere fluorescent in situ hybridization (cenM-FISH). RESULTS: Associations between the quadrivalent and the sex body or other autosomes were seen only in the t(13;20) carrier. Heterosynapsis was observed only in the t(10;14) carrier. Synaptic pairing abnormalities were seen in 71% of the spreads in the t(13;20) carrier and 30% of the spreads in the t(10;14) carrier. Recombination frequency was decreased in the t(13;20) carrier, but not in the t(10;14) carrier. CONCLUSIONS: By comparing these two different translocation carriers with different fertility outcomes, we discuss the possible mechanisms by which translocations might cause the spermatogenesis process to fail.

Key words: heterosynapsis/meiosis/meiotic checkpoints/reciprocal translocation/sex body

Introduction

Reciprocal translocations are among the most frequent chromosomal aberrations found in humans (De Braekeleer and Dao, 1991). Studies in infertile men have demonstrated a significantly higher frequency of reciprocal translocations than in controls (Shi and Martin, 2001). The effects of reciprocal translocations on the carriers depend on whether they are present in a balanced or in an unbalanced form. While unbalanced forms of reciprocal translocations usually cause mental retardation and physical problems, balanced forms do not have any phenotypic effect on the carriers, except that they may show variable sperm counts, ranging from normal counts to a low sperm count (oligospermia) or even a total absence of sperm in the ejaculate (azoospermia) (Van Assche et al., 1996). Even if the sperm count is not reduced, reciprocal translocation carriers have a risk of pre- and post-implantation losses or abnormal pregnancy outcome, due to the production of sperm with chromosomal imbalances (reviewed by Morel et al., 2004).

In order to pair during meiosis, translocated chromosomes and their homologues align themselves in a cross shape known as a quadrivalent. As meiosis progresses, the quadrivalent can segregate during anaphase I in several different ways. The meiotic behaviour of the quadrivalent will, first, determine the presence or absence of sperm in the ejaculate, and the proportions of balanced and unbalanced gametes if sperm is present (Oliver-Bonet et al., 2004). Some meiotic studies of infertile men carrying chromosomal rearrangements have suggested that the meiotic failure observed in these patients might be linked to an interaction between the sex body (SB) and the rearrangement (Gabriel-Robez et al., 1986). Others have suggested that such an association is not the main cause of the spermatogenic arrest (Chandley et al., 1986; Navarro et al., 1991), and propose the presence of pairing disturbances as a possible trigger of the germ cell destruction.

Different translocations behave uniquely (Rickards, 1983) and very little is known about the reasons for this particular behaviour. Factors such as the chromosomes involved in the reorganization, the size of the translocated and the interstitial segments, and the presence or absence of recombination foci are said to be determinant in the meiotic behaviour of reciprocal translocations. Meiotic chromosome analyses have been performed on infertile carriers, providing information about quadrivalent behaviour in these patients (Chandley et al., 1986; Gabriel-Robez et al., 1986; Paoloni-Giacobino
et al., 2000). However, until now, a comparison between azoospermic and normozoospermic translocation carriers has never been made, and the ability to assess recombination foci in prophase has not been possible. In this study, meiotic and recombination analysis in two cases of reciprocal translocations involving an acrocentric chromosome in two men with different fertility outcomes are reported and compared, in order to investigate the mechanisms by which translocations can interfere with the meiotic process.

Materials and methods

The study was performed on testicular biopsy samples from two different translocation carriers. One carried a constitutional 46,XY,t(10;14)(q24;q32) reciprocal translocation and was ascertained because of repeated reproductive failures. He was 27 years old at the time of sample collection. His semen parameters were found to be within the normal range. Meiotic analysis of this patient was performed at the Universitat Autònoma de Barcelona. The second carrier was referred for primary infertility. Analysis of his semen showed that no sperm were present, and his FSH level was 9 mU/mL. Karyotype analysis of peripheral blood lymphocytes, performed by classical G-banding and fluorescence in situ hybridization (FISH) techniques, revealed a constitutional reciprocal translocation 46,XY,t(13;20)(p11.1;p11.1). This patient was 39 years old at the time of sample collection. Meiotic analysis of this patient was performed at the University of Calgary by the same investigator, using the same procedures as those used for the previous translocation carrier. Configurations of the quadrivalents showing the position of the breakpoints of both reciprocal translocations are shown in Figure 1. Written consent was obtained from all patients and the study was approved by the University of Calgary institutional review board and by the Universitat Autònoma de Barcelona institutional ethics committee.

Fixation, spreading and immunocytotherapy of spermatocytes have been described elsewhere (Sun et al., 2004a). The primary antibodies used in this work to detect the centromeres, the recombination foci and the synaptonemal complexes (SCs) were, respectively: human CREST antibody (a gift from M. Fritzler, University of Calgary), rabbit MLH1 (Oncogene, San Diego, CA), goat SCP3 (a gift from T. Ashley, Yale University) and mouse SYN1 (a gift from P. Moens, York University) for the sample processed at the University of Calgary. Human CREST antibody (a gift from W. Earnshaw, University of Edinburgh, UK), mouse MLH1 antibody (Pharmingen; San Diego, CA) and rabbit SCP3 antibody (a gift from C. Heyting; University of Wageningen, The Netherlands) were used for the sample processed at the Universitat Autònoma de Barcelona. The primary antibodies were detected using a cocktail of blue, green and red fluorescence secondary antibodies: AMCA (Jackson Immunoresearch, West Grove, PA), Alexa 488 (Molecular Probes, Eugene, OR), fluorescein isothiocyanate (FITC; Sigma, Madrid, Spain), Alexa 555 (Molecular Probes), Cy3 (Jackson Immunoresearch) and tetramethylrhodamine isothiocyanate (TRITC; Sigma).

Each slide was scanned using a fluorescent photomicroscope (Zeiss Axiophot at the University of Calgary, and Olympus Bx60 at the Universitat Autònoma de Barcelona) equipped with the appropriate filter sets. Images of pachytene nuclei were captured and processed using Applied Imaging Cytovision 3.1 software (Applied Imaging Corporation, Santa Clara, CA) at the University of Calgary, and a Power Macintosh G3 with Smartcapture software (Digital Scientific; Cambridge, UK) at the Universitat Autònoma de Barcelona. Immunocytogenetic images of the SC were analysed to assess the number of MLH1 sites on each individual SC and in the whole cell, and the pachytene stage according to SB morphology (Solari, 1980).

After immunocytogenetic analysis was completed, multi-centromere FISH (cenM-FISH) (Nietzel et al., 2001) was performed on SC spreads following a protocol described elsewhere (Oliver-Bonet et al., 2003) with some modifications (Sun et al., 2004a). CenM-FISH was performed successfully on the t(10;14) carrier and on three controls. All three controls were testicular cancer patients who had undergone orchietomies, recruited from urology clinics. Unexpected susceptibility in the t(13;20) carrier to overdecondensation of the chromatin under normal conditions made cenM-FISH analysis impossible for this patient. CenM-FISH images for the t(10;14) carrier and the controls were captured and individual bivalents were identified in the same cells that had been analysed previously. The position of the centromeres of the acrocentric chromosome 13 and the metacentric chromosome 20 allowed the localization of these chromosomes within the quadrivalent in the t(13;20) carrier.

Results


t(10;14)

One hundred pachytene cells were analysed; results for the t(10;14) translocation carrier are summarized in Table I. The number of recombination foci (MLH1) ranged from zero to five per bivalent and from 38 to 59 per cell. The mean overall frequency of MLH1 sites for this patient was 50.01%, with an SD of ±4.6. Synaptic abnormalities (unpaired regions), SC discontinuities (gaps) or both were seen in 30% of the analysed cells.

All observed quadrivalents displayed a closed ring configuration (Figure 2a, b and c). No quadrivalent was present in 9% of the spreads analysed. The percentage of cells with synaptic adjustment (heterologous synapsis) increased through the pachytene stages from 11.1% at early pachytene to 77.8% at late pachytene. An association between the quadrivalent and the SB was never found in this patient. The number of MLH1 foci within the quadrivalent ranged from three to six. Three, four, five and six MLH1 foci were found in 3.3, 19.8, 68.1 and 7.8% of quadrivalents, respectively (Table I). Mean recombination frequencies were calculated for each arm of the quadrivalent (Table II). The results obtained were compared with data obtained from three control donors with normal karyotypes. A significant increase in the recombination frequency was observed in chromosome 10q (Student t-test, P < 0.001).

Fifty cells from the t(10;14) carrier were analysed using cenM-FISH (Figure 2d). Each bivalent was identified (Figure 2e) and the mean number of MLH1 per bivalent was scored and compared with control data (Table III). As shown by recombination analysis of the quadrivalent (above),
the recombination frequency in chromosome 10 was significantly increased (Student $t$-test, $P < 0.001$); there were no significant differences found in other bivalents.

Table I. Recombination and synapsis analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>MLH1 foci</th>
<th>Synaptic anomalies $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foci/quadrivalent (%)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Control $^b$</td>
<td>0 1 2 3 4 5 6</td>
<td>48.0 ± 4.7</td>
</tr>
<tr>
<td>t(10;14)</td>
<td>0 1 2 3 4 5</td>
<td>50.0 ± 4.6</td>
</tr>
<tr>
<td>t(13;20)</td>
<td>1.0 7.0 14.0 56.0 22.0 0</td>
<td>45.4 ± 7.6*</td>
</tr>
</tbody>
</table>

$^a$Regions of the SC which were unpaired or showed gaps.
$^b$Sun et al. (2004b).
$^*P = 0.001$.
$^{**}P < 0.0001$.

Figure 2. Immunostained images of SCs in translocation carrier t(10;14): SCP3 and SYN1 are stained in red, MLH1 is stained in green. (A) Quadrivalent showing heterosynapsis, with a recombination MLH1 focus at the chromosome 14 breakpoint (arrow). (B) Quadrivalent showing heterosynapsis, with no apparent suppression of recombination in the quadrivalent. (C) Quadrivalent showing asynapsis in the vicinity of the breakpoints, with a recombination MLH1 focus at the chromosome 10 breakpoint (arrow). (D) CenM-FISH signals in a pachytene cell. (E) Immunocytogenetic staining and identification of all bivalents in the same pachytene cell shown in (D), with complete separation of chromosomes 10 and 14.

t(13;20)

One hundred pachytene cells were analysed; results for this translocation carrier are summarized in Table I. The number of recombination foci (MLH1) ranged from zero to five per bivalent and from 15 to 62 per cell. The mean overall frequency of MLH1 sites for this patient was 45.4%, with an SD of ± 7.6, significantly different from control values (Student $t$-test, $P = 0.001$) (Sun et al., 2004b). Synaptic abnormalities (unpaired regions), SC discontinuities (gap) or both were seen in 71% of the analysed cells, which is significantly different from controls (Student $t$-test, $P < 0.0001$) (Sun et al., 2004b).

Almost all quadrivalents configurations found in the t(13;20) carrier were chain open at 13p (98%), and 65% displayed significant asynapsed regions in both arms of chromosome 20 (Figure 3a). Closed configurations were found in only two cells; although they displayed apparent synapsis at this point, these configurations did not have an MLH1 focus on 13p (Figure 3b). The number of MLH1 foci located on the quadrivalents ranged from zero to four, with only 1% lacking an MLH1 focus. There were one, two, three and four MLH1 foci in 7, 14, 56 and 22% of the quadrivalents, respectively (Table I). Mean recombination frequencies were calculated for each arm of the quadrivalent (Table II). The results obtained were compared with data obtained from
three control donors with normal karyotypes. We observed a significant decrease in recombination frequency in chromosome 13q (Student t-test, \( P < 0.0001 \)) and in chromosome 20q (Student t-test, \( P = 0.0015 \)). An association of the quadrivalent with the SB, a G-group chromosome or both was found in 46, 3 and 2% of the analysed cells, respectively (Table IV) (Figure 3c–f). The percentage of cells containing a quadrivalent associated with the SB increased as the pachytene stage progressed: 26.5% at early pachytene, 75% at mid-pachytene and 95% at late pachytene. The proportion of cells at the early pachytene stage was high when compared with control individuals (de Boer et al., 2004): 68 versus 33%, and late pachytene stage cells were found in a low proportion of cells (20 versus 60%).

### Discussion

The analysis performed showed different meiotic behaviours for the two carriers. Whereas the t(10;14) (normozoospermic) carrier displayed heterosynapsis, a normal percentage of asynapsis and recombination foci, and no association of the quadrivalent with the SB, the t(13;20) (azoospermic) carrier showed a high proportion of cells with asynapsis, SB association and a low recombination frequency.

According to the R-band/G-band model (Ashley, 1988), synopsis in translocations with both breakpoints in G-negative bands is limited to homology, and recombination frequency is normal. Further, translocations with both breakpoints on the edge of a G-negative band, next to a G-positive band display different patterns of meiotic behaviour: translocations with suppression of recombination are expected to display heterologous synopsis, while translocations with normal recombination show only homologous synopsis. The synaptic behaviour of the t(10;14) translocation carrier does not fit this model, although both breaks in the t(10;14) carrier are in G-negative bands adjacent to G-positive bands (Figure 1). Heterologous pairing has been found in this patient, but there is no apparent suppression of recombination in the vicinity of the break points (Figure 2a and c).

The t(13;20) translocation carrier has both breaks on G-negative bands, close to the centromeres. According to the G-band position model, only homologous pairing is allowed (no heterologous synopsis), and there is indeed no heterologous synopsis and no suppression of recombination in the vicinity of the break points (13p and 20p) seen in this patient. There is, however, a suppression of recombination within the quadrivalent at 13q and 20q (the two arms not implicated in the reorganization), but this may be more related to the general decrease in recombination observed in this patient.

A gradual increase in quadrivalent–SB association through the meiotic prophase has been described in other reciprocal and Robertsonian human translocation carriers (Gabriel-Robez et al., 1986; Navarro et al., 1991; Yu et al., 1995). Different hypotheses have been suggested in order to explain the failure of the meiotic process as a result of this association. According to an early model (Lifschytz and Lindsley, 1972), the association would interfere in the normal sex chromosome inactivation, reversing this process and allowing inappropriate expression of genes located in the X chromosome. An alternative explanation of the spermatogenic arrest, the spreading of the X chromosome inactivation to associated autosomes, was proposed by Jaafar et al. (1989). Our analysis shows a normal timing and progression of condensation through the pachytene stage for the SB in both carriers. Since XY condensation has been related to SB inactivation (Fernandez-Capetillo et al., 2003), it appears that the association of the quadrivalent with the SB does not trigger the activation of X genes in the two translocation carriers studied in this work.

The normal proportion of pre-pachytene stage cells and the abnormal proportions of cells in the early and late pachytene
stages in the t(13;20) carrier indicate the presence of at least two different meiotic checkpoints. We have observed that unpaired regions within the quadrivalent show a tendency to pair with each other when heterologous pairing is allowed by synaptic adjustment. If there is no heterologous pairing, an association of the quadrivalent with the SB may eventually occur. The fact that the association of the quadrivalent with the SB also increases through pachytene stage, and that decreased numbers of cells reach the later pachytene stages may indicate the presence of an early–mid pachytene checkpoint acting on asynapsed segments, deleting the cells containing this pairing abnormality. In addition to this first pachytene checkpoint, our data indicate the presence of another spermatogenic arrest occurring later in the meiotic process, at a post-synaptic stage. This second breakdown of the meiotic process could be caused by an inactivation of genes located on the regions associated with the SB (Solari, 1999). This gene inactivation would block transcription of some of these genes, which in turn could trigger an apoptotic response. Alternatively, the association of the SB with the quadrivalent could be detected at a checkpoint that spots abnormalities in the bivalent alignment on the meiotic spindle, stopping the meiotic process. In either case, cells surviving the first checkpoint would be eliminated later, resulting in the azoospermia observed in this t(13;20) carrier.

Female carriers of reciprocal translocations who take part in assisted reproduction techniques show a poor reproductive outcome (Gekas et al., 2001). However, this failure in reproduction is not usually because of a lack of oocytes, but because of a low pregnancy rate, mainly due to the presence of chromosomal imbalances (Pujol et al., 2003). The presence of oocytes in female carriers of reciprocal translocation indicates that oogenesis is not dramatically interrupted as it is in azoospermic male carriers. It has been observed that the presence of an abnormality during spermatogenesis may cause meiotic arrest, whereas the same abnormality in female meiosis may not stop the process, allowing the formation of aneuploid gametes (Hunt and Hassold, 2002). Thus, the differences in response to abnormal situations may explain why the same chromosomal reorganization may cause male spermatogenic failure, yet allow oogenesis to progress in female carriers (Paoloni-Giacobino et al., 2000).

It has also been proposed that the type of chromosomes involved in the translocation is of the utmost importance for the carrier’s fertility. Thus translocations involving an acrocentric chromosome have been thought to be more deleterious for the fertility of the carrier than translocations not involving acrocentric chromosomes (Gabriel-Robez et al., 1986), because of the tendency of acrocentric chromosomes to associate with the SB. However, no quadrivalent–SB association was found in our t(10;14) carrier, showing that the presence of an acrocentric chromosome in the translocation does not necessarily induce a quadrivalent–SB association. Moreover, association with the SB in the t(13;20) carrier was initiated mainly at the asynapsed regions located in bivalent 20/der(20): either 20p, 20q or 13p (Figure 3e and f).

Interchromosomal effects have been defined as disturbances caused by a chromosomal reorganization on the pairing and synopsis of the chromosomes unrelated to the rearrangement (Lejeune, 1963). At the pachytene level, these disturbances could be detected as meiotic impairments (asynapsis) or recombination defects. CenM-FISH results on the t(10;14) carrier show that recombination is not affected in chromosomes unrelated to the translocation. In addition, the presence of pairing defects is not very different from that observed in controls (Sun et al., 2004b). Thus, in this carrier, the presence of the translocation does not affect normal synopsis.
recombination and segregation of the other chromosomes. Decondensed sperm nuclei from this patient had been analysed previously for aneuploidy, and no apparent interchromosomal effect was reported (Oliver-Bonet et al., 2004). As no cenM-FISH results were obtained from the t(13;20) carrier, no comparison can be made regarding the recombination rate of individual bivalents. Nevertheless, overall recombination was significantly decreased and the presence of synopsis anomalies was significantly high. Moreover, the presence of an association of the quadrivalent (13p arm, asynapsed) with a G group chromosome was observed in 5% of the cells (Figure 3d and e). As this patient was azoospermic, no sperm aneuploidy assay was performed. However, abnormalities found at the pachytene stage probably do represent an actual interchromosomal effect. These results agree with observations made by Vegetti et al. (2000) and Pellestor et al. (2001): that interchromosomal effects would be restricted to translocation carriers with poor or abnormal semenograms.

In conclusion, we propose that factors related to the position of the breakpoints, the size and characteristics of the interstitial segments and the presence or absence of recombination foci are responsible for synopsis failure within the quadrivalent. The presence of these asynapsed regions is responsible for spermatogenic breakdown, either by the detection of these asynapsed regions by pachytene check-points, or through the association between the quadrivalent and the SB.

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