Silencing of unpaired meiotic chromosomes and altered recombination patterns in an azoospermic carrier of a t(8;13) reciprocal translocation

Kyle A. Ferguson, Victor Chow and Sai Ma

Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, Canada

1Correspondence address. Department of Obstetrics and Gynaecology, Room D414B, BC Women’s Hospital and Health Centre, University of British Columbia, D6-4500 Oak Street, Vancouver, British Columbia, Canada V6H 3N1 Tel: +1-604-875-2345 ext. 5686; Fax: +1-604-875-2722; E-mail: sai@interchange.ubc.ca

BACKGROUND: Male carriers of structural chromosomal abnormalities provide a useful model for studying the effects of impaired synapsis on human meioses and male fertility. METHODS: We used immunofluorescent techniques to examine recombination (MLH1), synapsis (SYCP3/SYCP1) and transcriptional inactivation (BRCA1/γH2AX/RNA polymerase II) of meiotic chromosomes in an azoospermic carrier of a t(8;13) reciprocal translocation. Two biopsies were performed 1 year apart and on different testes. RESULTS: Global recombination rates differed between the two biopsies. Although global recombination rates were not altered when compared with control men, recombination frequencies were reduced specifically on the rearranged chromosomes. Asynapsed quadrivalents were observed in 90% and 87% of pachytene nuclei from the first and second biopsies, respectively, and were frequently associated with the sex chromosomes. BRCA1 and γH2AX, two proteins implicated in meiotic sex chromosome inactivation, localized along asynapsed regions regardless of whether or not they were associated with the sex chromosomes. Immunostaining for RNA polymerase II provided further evidence that unsynapsed regions are silenced during human meiosis. CONCLUSIONS: The fidelity of synapsis is a critical factor in determining the outcome of gametogenesis in humans, as the transcriptional inactivation of asynapsed regions may silence meiotic genes, leading to meiotic arrest and infertility.

Keywords: meiosis; recombination; meiotic silencing of unsynapsed chromatin; chromosomal rearrangements; male infertility

Introduction

Reciprocal translocations are present in 0.1% of phenotypically normal newborns; however, the incidence of reciprocal translocations among infertile men is approximately 10 times greater (De Braeckeleer and Dao, 1991). During spermatogenesis in carriers of balanced translocations, sperm with unbalanced chromosomal complements can be produced. The proportion of chromosomally unbalanced sperm produced by translocation carriers can range from 19% to more than 80%, and appears to be dependent on the translocation (reviewed in Benet et al., 2005). The increase in chromosomally abnormal sperm can lead to an increased risk of pre- and post-implantation pregnancy loss. Furthermore, if a fetus with an unbalanced chromosomal complement survives to term, it is likely to suffer from mental retardation or congenital malformations. Although the greater risk of pregnancy loss contributes to the decreased fertility among translocation carriers, spermatogenesis may also be directly impaired. Sperm parameters are highly variable among translocation carriers, ranging from normozoospermic to a complete absence of sperm in the ejaculate, and are likely dependent on the chromosomes involved and the sites of the breakpoints.

During meiosis, homologous chromosomes undergo synapsis, in which a protein structure known as the synaptonemal complex forms between the paired chromosomes, and meiotic recombination, in which genetic material is exchanged between the chromosomes. These two events are critical to the fidelity of meiosis, and failures in both of these processes have been associated with meiotic arrest and infertility (Gonsalves et al., 2004) and may also contribute to the production of aneuploid sperm (Ma et al., 2006; Ferguson et al., 2007). In order to align their homologous regions during synapsis, reciprocal translocations adopt a quadrivalent structure. The meiotic behavior of the quadrivalent is thought to have a significant impact on the fertility status of the carrier, and may explain the variation in sperm parameters among male carriers. Meiotic studies on infertile male carriers of reciprocal translocations have shown that the regions surrounding the breakpoints often fail to completely synapse (Chandley et al., 1986; Gabriel-Robez et al., 1986; Johannisson et al., 1987;
et al., 2005a). Furthermore, these asynapsed regions have been found to associate with the sex chromosomes. During the male meiosis, the X and Y chromosomes are transcriptionally silenced, forming a condensed sex body. Associations between quadrivalents and the sex body in fertile men have lead to the hypothesis that sex chromosome inactivation may spread to the associated regions of autosomal chromosomes, leading to cell death (reviewed in Oliver-Bonet et al., 2005b).

Meiotic sex chromosome inactivation (MSCI) is characterized by the localization of phosphorylated histone H2AX on the sex chromosomes, which is thought to be critical for chromatin condensation and transcriptional inactivation (Celeste et al., 2002). BRCA1 is also critical for MSCI, recruiting ATR (ataxia telangiectasia and RAD3 related) to phosphorylate H2AX (Xu et al., 2003; Turner et al., 2004). Recent studies, however, have suggested that the phenomenon of meiotic inactivation may not be limited to the sex chromosomes: unsynapsed autosomal chromosomes have been shown to undergo a similar transcriptional silencing in the germ cells of mice (Baarends et al., 2005; Turner et al., 2005). Nevertheless, there is little information on the inactivation of unpaired meiotic chromosomes in humans. Carriers of structural chromosomal abnormalities generally display disturbed homologous synapsis, and thus provide an exceptional model for studying the behavior of unpaired autosomal chromosomes during human meiosis. Here, we present an analysis of synapsis, recombination and transcriptional silencing in spermatocytes from an azoospermic carrier of a t(8;13) reciprocal translocation.

Materials and Methods

A 45-year-old male had a 5 year history of infertility. Semen analysis showed an absence of sperm in the semen sample, hormonal profiles were normal and a physical examination did not reveal any obstruction in the reproductive tract. Routine karyotyping showed that the man was a heterozygous carrier of a t(8;13)(q21.p11) reciprocal translocation. Two testicular biopsies were performed one year apart and on separate tests in order to extract sperm for ICSI. A few sperm were found from the first biopsy, and none from the second. A histological analysis found germ cell maturation arrest. A small portion of testicular tissues from both biopsies were used for the meiotic analyses reported in this study. As a control, testicular tissue samples were retrieved during vasectomy reversals on eight proven fertile 46,XY men. Ethical approval was obtained from the University of British Columbia Ethics Committee before initiating this study.

Meiotic analyses

Testicular tissue was processed according to the methods reported by Barlow and Hulten (1998). A primary antibody cocktail [rabbit anti-MLH1 (Oncogene, San Diego, CA, USA), 1:37.5; mouse anti-SYCP3 (provided by P. Moens, York University), 1:300; mouse anti-SYCP1 (provided by P. Moens), 1:300 and human CREST antisera, 1:25] was applied to slides and incubated at 37°C overnight. A secondary antibody cocktail [FITC Donkey antirabbit IgG (Jackson Immuno-Research, West Grove, PA, USA) 1:50, TRITC Goat antirabbits IgG (Jackson ImmunoResearch) 1:100, AMCA Donkey antihuman IgG (Jackson ImmunoResearch) 1:50, 1X antibody diluting buffer (ADB)] was applied and slides were incubated in a 37°C humid chamber for 90 min. Slides were scanned with a Zeiss Axioplan epifluorescent microscope equipped with appropriate filters. Images of the SYCP3/ SYCP1 fragments, MLH1 and CREST sites were captured using Cyto-vision V2.81 Image Analysis software (Applied Imaging International, San Jose, CA, USA). One hundred pachytene cells were captured for each biopsy, cell co-ordinates were recorded and printouts were analyzed for the number of recombinant foci and synaptic errors.

Fluorescence in situ hybridization (FISH) was performed on the slides that had been previously immunostained, to identify the arms of the translocated chromosomes. Coverslips were removed and the slides were washed in PBS for 5 min, followed by dehydration in an ethanol series (70%, 80%, 90% and 100%). After drying, a probe mixture of LSI 13 (13q14) SpectrumGreen, CEP 8 SpectrumOrange and 8q24 LSI MYC Dual Color (SpectrumOrange/SpectrumGreen) (Vysis Inc., Downer Grove, IL, USA) was added to the slides. Slides were co-denatured on a hotplate at 75°C for 5 min, then incubated overnight in a humid chamber. Coverslips were removed, and slides were washed in 0.4XSSC/0.3%NP-40 at 72°C for 2 min, followed by 2XSSC/0.1%NP-40 for 30 s at room temperature. After air drying in the dark, antifade and coverslips were added. Cells were then relocated and FISH signals were captured.

Following FISH analysis, a second round of immunostaining was performed on the same slides. FISH probes were washed off by rinsing the slides in phosphate-buffered detergent (PBD) for 5 min, followed by an ethanol series (70%, 80%, 90% and 100%). A primary antibody cocktail of mouse anti-γH2AX (Upstate Biotech, Lake Placid, NY, USA) at 1:1000 dilution and rabbit anti-BRCA1 (Santa Cruz Biotech, CA, USA) at 1:50 dilution was applied to slides. This was followed by a secondary antibody cocktail of FITC Donkey antirabbit IgG (Jackson ImmunoResearch) at a dilution of 1:100 and TRITC Goat antirabbits IgG (Jackson ImmunoResearch) at a dilution of 1:100. Cells were relocated and the γH2AX and BRCA1 localization was observed.

To observe transcriptional activity in the pachytene nuclei, an unused slide from the second biopsy on the t(8;13) patient was immunostained with a primary antibody cocktail of mouse anti-RNA polymerase II (8wg16, Abcam, Cambridge, MA, USA) at 1:50 dilution, rabbit anti-SYCP3 (P. Moens) at 1:50 dilution, rabbit anti-SYCP1 (P. Moens) at 1:50 dilution and CREST antisera at 1:25 dilution. The secondary antibody cocktail contained FITC Donkey antirabbit IgG (Jackson ImmunoResearch) 1:50, TRITC Goat antirabbits IgG (Jackson ImmunoResearch) 1:100, AMCA Donkey antihuman IgG (Jackson ImmunoResearch) 1:100.

Statistical analysis

The Mann–Whitney test was used to compare mean rates of genome-wide recombination between the biopsies. The Fisher’s exact test was used to compare the frequency of MLH1 foci on arms of the translocated chromosomes.

Results

Genome-wide and chromosome-specific recombination frequencies

One hundred pachytene nuclei were analyzed from each biopsy after immunostaining to observe the SC and sites of crossover (Fig. 1A). The mean rates of recombination were 47.3 and 49.6 crossovers per cell in the first and second biopsies, respectively (Table I). Recombination was also assessed in pachytene nuclei from eight fertile control men (Table I). Mean rates of recombination ranged from 44.3 to 51.3 crossovers per cell in the control men. Genome-wide recombination was significantly
were also interested in quantifying recombination specifically on the translocated chromosomes. Combining immunofluorescent analyses with FISH allowed us to identify the chromosome arms of the quadrivalent (Fig. 1B). Recombination on chromosome 13 was examined in 50 and 91 pachytene nuclei from the first and second biopsies on the t(8;13) patient, as well as in 338 pachytene nuclei from six control men (Table II). In the control population, 13q displayed two MLH1 foci in 83.4% of pachytene spermatocytes, which was significantly greater than 48.0% and 48.4% of pachytene nuclei from the first and second biopsies, respectively (P < 0.01, Fisher’s test). Recombination on chromosome 8 was examined in 158 spermatocytes from two control men, as well as 50 and 91 spermatocytes from the first and second biopsies (Table II). There was no difference in the frequency of crossovers on the 8p arm of the t(8;13) quadrivalent when compared with the control men. However, the 8q arm displayed double crossovers in 36.7% of pachytene nuclei from the control men, which was significantly greater than 20.0% (P = 0.037, Fisher’s test) and 19.8% (P = 0.006, Fisher’s test) in the first and second biopsies, respectively (Table II). Spermatocytes from the first biopsy on the t(8;13) carrier displayed achiasmate 8q arms in 6.0% of nuclei, which was significantly greater than 0.6% of nuclei from the control men (P = 0.044, Fisher’s test).

**Synapsis, XY-association and inactivation of quadrivalents**

In order to pair homologous regions, the chromosomes involved in a reciprocal translocation adopt a quadrivalent configuration during meiosis (Fig. 2A and B). The t(8;13) carrier displayed a high degree of asynapsis in the chromosomal regions adjacent to the breakpoints, with 90% and 87% of quadrivalents from the first and second biopsies, respectively, displaying asynapsed quadrivalents (Table III). These asynapsed quadrivalents were found to associate with the sex chromosomes (Fig. 2C) in 74% of pachytene nuclei from both biopsies (Table III). Only 10% and 13% of quadrivalents in the first and second biopsy, respectively, displayed heterosynapsis which enabled the quadrivalents to fully synapse, and these configurations displayed no association with the sex chromosomes (Fig. 2D).

Spermatocytes that were previously immunostained to observe synapsis and recombination were subsequently immunostained to investigate the localization of BRCA1 and γH2AX, two proteins implicated in MSCI (Fig. 3). Pachytene nuclei from normal 46,XY men displayed BRCA1 and γH2AX localization exclusively on the sex chromosomes, indicating their transcriptional inactivation during meiosis (Fig. 3A and A’). BRCA1 was observed only on the asynapsed axial elements of the sex chromosomes, but not on the paired pseudoautosomal region (arrow, Fig. 3A’). Asynapsed regions of the quadrivalent that were associated with the sex chromosomes were positive for both BRCA1 and γH2AX, suggesting that these regions may be inactivated during meiosis (Fig. 3B and B’). However, association of unpaired autosomal elements with the sex chromosomes does not appear to be necessary for transcriptional inactivation. Asynapsed quadrivalents displaying no association with the sex chromosomes were also different between the two biopsies, with the rate of recombination significantly greater in the second biopsy (P = 0.0134; Mann–Whitney test). However, mean recombination rates in both biopsies were within the range of recombination rates observed in the control population. Recombination between the sex chromosomes also appeared to be unaffected in the t(8;13) carrier, with recombination occurring in 82.0% and 78.0% of spermatocytes in the first and second biopsies, respectively, which were both within the range observed in the control men (Table I). Furthermore, 4.0% and 2.0% of nuclei in the first and second biopsies, respectively, showed at least one achiasmate autosomal bivalent, which were not beyond the range observed in the control men (Table I).

Although genome-wide recombination and recombination between the sex chromosomes appeared to be unaffected, we

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**Figure 1:** Immunofluorescent and FISH analysis of spermatocytes from a t(8;13) carrier.

(A) Spermatocytes were immunostained to visualize the synaptonemal complex (SYCP3/SYCP1; red), MLH1 foci (green) and centromeres (blue). (B) FISH was performed to identify the centromere of chromosome 8 (red), 8q24 (red/green) and 13q14 (green). In (A), a quadrivalent displays complete synapsis and no association with the sex chromosomes (arrow head).
positive for BRCA1 and γH2AX (Fig. 3C and C’). Quadrivalents displaying heterosynapsis and complete pairing of the chromosomes showed no γH2AX staining, and BRCA1 did not appear to be localized along chromosome arms (Fig. 3D and D’).

To further investigate the transcriptional silencing of unsynapsed regions, we immunostained new slides with antibodies against RNA polymerase II and SYCP3 (Fig. 4). Pachytene nuclei from control men display an absence of RNA polymerase II in the region surrounding the transcriptionally silenced sex chromosomes (Fig. 4A). Pachytene nuclei in which the t(8;13) quadrivalent was associated with the sex chromosomes also displayed an absence of RNA polymerase II from the sex chromosomes, as well as the attached asynapsed autosomal chromosomes (Fig. 4B). Similarly, asynapsed quadrivalents that were not directly associated with the sex chromosomes were found to be negative for RNA polymerase II, suggesting that they are also transcriptionally silenced (Fig. 4C).

Discussion

Impaired recombination on translocated chromosomes

To date, recombination frequencies have been studied in four carriers of structural chromosomal abnormalities using immunofluorescent techniques to visualize MLH1 foci (Table III). Only one of the translocations studied so far, an azoospermic t(Y;1) carrier, displayed a rate of genome-wide recombination that was outside the range observed in control men (Sun et al., 2005a). Three autosomal–autosomal reciprocal translocations (Oliver-Bonet et al., 2005a; Pigozzi et al., 2005) displayed rates of recombination that were similar to those observed in normal 46,XY men (Hassold et al., 2004; Sun et al., 2005b, 2006). Similarly, global-recombination rates did not appear to be altered in both biopsies from the t(8;13) carrier that we examined. Interestingly, global-recombination in the second biopsy was significantly greater than the first biopsy. Although recombination has been shown to be highly variable between individuals (Hassold et al., 2004; Sun et al., 2005b, 2006), little is known regarding the variability within an individual. This analysis of recombination in two sites from the same male is the first evidence, to our knowledge, that global-recombination rates are variable within the same individual. The first biopsy was taken from the left testes and, a year later, a second biopsy was performed on the right testes. Thus, due to differences in both the site of tissue retrieval and the time of the biopsies, we cannot attribute the variability to differences between the testes, or to environmental factors that may have changed over time. Recombination plays an important role in ensuring the proper segregation of chromosomes during meiosis, and the temporal variation in recombination rates may explain the variation seen in our study.

Table I. Analysis of MLH1 foci in an azoospermic carrier of a t(8;13) translocation.

<table>
<thead>
<tr>
<th>Control men</th>
<th># of cells analyzed (n)</th>
<th>Mean no. of recombination foci per cell (95% CI)</th>
<th>Range of recombination foci</th>
<th>Recombination in XY bivalent (%)</th>
<th>Proportion of cells with an autosomal bivalent lacking an MLH1 focus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>100</td>
<td>48.0 (47.0–48.9)</td>
<td>39–56</td>
<td>81.0</td>
<td>3.0</td>
</tr>
<tr>
<td>C4</td>
<td>30</td>
<td>47.3 (45.5–49.1)</td>
<td>37–57</td>
<td>80.0</td>
<td>3.3</td>
</tr>
<tr>
<td>C5</td>
<td>41</td>
<td>45.2 (43.9–46.6)</td>
<td>37–53</td>
<td>80.5</td>
<td>2.4</td>
</tr>
<tr>
<td>C6</td>
<td>46</td>
<td>48.6 (47.4–49.9)</td>
<td>39–58</td>
<td>78.3</td>
<td>0</td>
</tr>
<tr>
<td>C7</td>
<td>41</td>
<td>46.2 (44.5–47.8)</td>
<td>34–59</td>
<td>92.7</td>
<td>0</td>
</tr>
<tr>
<td>C8</td>
<td>100</td>
<td>51.3 (50.2–52.4)</td>
<td>30–66</td>
<td>87.0</td>
<td>3.0</td>
</tr>
<tr>
<td>C9</td>
<td>101</td>
<td>44.3 (43.2–45.5)</td>
<td>28–55</td>
<td>71.0</td>
<td>5.0</td>
</tr>
<tr>
<td>C10</td>
<td>101</td>
<td>48.3 (47.2–49.5)</td>
<td>31–61</td>
<td>76.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Mean</td>
<td>47.4</td>
<td>80.8</td>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(8;13) biopsy 1</td>
<td>100</td>
<td>47.3 (46.0–48.6)*</td>
<td>24–56</td>
<td>82.0</td>
<td>4.0</td>
</tr>
<tr>
<td>t(8;13) biopsy 2</td>
<td>100</td>
<td>49.6 (48.5–50.7)*</td>
<td>26–64</td>
<td>78.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*Recombination was significantly different between the two biopsies on the t(8;13) carrier (P = 0.013, Mann–Whitney test).

Table II. Analysis of crossovers on chromosome arms involved in the t(8;13) translocation.

<table>
<thead>
<tr>
<th># of cells</th>
<th>13q [% (n)]</th>
<th>8p [% (n)]</th>
<th>8q [% (n)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 foci 1 foci ≥2 foci</td>
<td>0 foci 1 foci ≥2 foci</td>
<td>0 foci 1 foci ≥2 foci</td>
</tr>
<tr>
<td>Control men</td>
<td>338</td>
<td>0</td>
<td>16.6 (56)</td>
</tr>
<tr>
<td>t(8;13) biopsy 1</td>
<td>50</td>
<td>4.0 (2)</td>
<td>48.0 (24)</td>
</tr>
<tr>
<td>t(8;13) biopsy 2</td>
<td>91</td>
<td>1.1 (1)</td>
<td>50.5 (46)</td>
</tr>
</tbody>
</table>

aP < 0.01 when compared with the pooled control men, Fisher’s test.
bP < 0.02 when compared with the pooled control men, Fisher’s test.
cP < 0.05, when compared with the pooled control men, Fisher’s test.
variation in sperm aneuploidy rates observed in the same men over time (Rubes et al., 2005).

FISH on spermatocytes allowed us to quantify recombination on the chromosome arms of the translocated chromosomes. Recombination frequencies on the 8p arm were not altered in either biopsy from the t(8;13) carrier when compared with the control men. However, recombination on the 8q and 13q arms was reduced in both biopsies from the t(8;13) carrier, with a greater proportion of quadrivalents having only a single crossover on these arms, as opposed to the double crossovers that were more common in the control men. It is likely that the high degree of asynapsis on these arms prevented the second crossover from occurring. Similarly, Pigozzi et al. (2005) reported a decrease in recombination and a change in the distribution of crossovers on the 14q arm in a t(11;14)(q13;q32) carrier, most likely due to asynapsis. Thus, although genome-wide recombination rates may be normal in translocation carriers, recombination may be disturbed specifically on the rearranged chromosomes.

Behavior of unpaired meiotic chromosomes

The fidelity of synapsis in male carriers of chromosomal rearrangements is thought to be critical for the completion of spermatogenesis. Unpaired chromosomal regions may be

Table III. Comparison of synapsis and recombination in carriers of different reciprocal translocations.

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Semen parameters</th>
<th>Mean recombination foci per cell</th>
<th>% of cells with asynapsed quadrivalents</th>
<th>% of cells with XY-quadrivalent associations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;13)(q21;p11)</td>
<td>Azoospermic</td>
<td>47.3 ± 6.5</td>
<td>90</td>
<td>74</td>
<td>Present study</td>
</tr>
<tr>
<td>biopsy 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(8;13)(q21;p11)</td>
<td>Azoospermic</td>
<td>49.6 ± 5.6</td>
<td>87</td>
<td>74</td>
<td>Present study</td>
</tr>
<tr>
<td>biopsy 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>Azoospermic</td>
<td>48.0 ± 3.5</td>
<td>98</td>
<td>20</td>
<td>Pigozzi et al. (2005)</td>
</tr>
<tr>
<td>t(13;20)(p11;p11)</td>
<td>Azoospermic</td>
<td>45.4 ± 7.6</td>
<td>71</td>
<td>46</td>
<td>Oliver-Bonet et al. (2005a)</td>
</tr>
<tr>
<td>t(Y;1)(q24;q32)</td>
<td>Normozoospermic</td>
<td>50.0 ± 4.6</td>
<td>30</td>
<td>0</td>
<td>Oliver-Bonet et al. (2005a)</td>
</tr>
<tr>
<td>t(Y;1)(q24;q32)</td>
<td>Azoospermic</td>
<td>34.9 ± 18.4</td>
<td>n/a</td>
<td>n/a</td>
<td>Sun et al. (2005a)</td>
</tr>
</tbody>
</table>
Asynapsed regions of the quadrivalents display BRCA1 and H2AX labeling along unsynapsed axes, H2AX phosphorylation and transcriptional silencing of BRCA1 along unsynapsed axes, H2AX phosphorylation and transcriptional silencing of BRCA1 and H2AX localization, regardless of whether or not they pair with the sex chromosomes. Conversely, quadrivalents displaying heterosynapsis and complete pairing display no clear BRCA1 or H2AX labeling. These observations were consistent with those of Sciurano et al. (2007), who recently found that the asynapsed regions of quadrivalents show both BRCA1 and H2AX localization. Immunostaining for RNA polymerase II provided further evidence that pairing with the sex chromosomes is not required for unsynapsed regions to be transcriptionally silenced. Thus, the hypothesis that MSCI spreads to the quadrivalent is not entirely correct. Although asynapsed quadrivalents associated with the sex chromosomes are silenced, pairing with the sex chromosomes does not appear to be required for this inactivation. These observations suggest that asynapsis is the driving force behind meiotic inactivation in humans, and is not specific to the sex chromosomes (Turner et al., 2006). Homolka et al. (2007) recently found that pachytenes spermatocytes of mice who carried a t(16;17) translocation showed a partial suppression of genes near the breakpoint on chromosome 17, providing evidence of MSUC at the mRNA level. Surprisingly, they also found an overexpression of genes on the X chromosome, suggesting that the XY-association of the quadrivalent may also contribute to male sterility by disrupting MSCI (Homolka et al., 2007).

Carriers of structural chromosomal abnormalities provide a useful model for studying the behavior of meiotic chromosomes when synopsis is compromised. Although global recombination rates do not appear to be dramatically altered in carriers of reciprocal translocations, recombination may be altered on the rearranged chromosomes, possibly due to the presence of large asynapsed regions. By performing biopsies on both testes of the same patient, we have provided evidence that recombination rates may differ either between the testes or may vary over time in the same individual. The azoospermic t(8;13) patient displayed a high frequency of asynapsis in the quadrivalent, providing further evidence that the fidelity of detection by a pachytenes checkpoint, leading to apoptotic death of the cell and spermatogenic arrest (Odorisio et al., 1998). Indeed, studies on azoospermic carriers of reciprocal translocations have reported a high degree of asynapsis around the breakpoints (Table III). The azoospermic t(8;13) carrier reported on in this study further supports the relationship between asynapsis and infertility, as the majority of quadrivalents from both biopsies failed to pair properly. These asynapsed regions were also found to migrate toward, and pair with the sex chromosomes during pachytenes. It has been suggested that the asynapsed regions of the quadrivalents attempt to pair with the sex chromosomes in order to avoid detection by the pachytenes checkpoint which monitors synopsis (Oliver-Bonet et al., 2005b). Although there is little information on the meiotic behavior of quadrivalents in normozoospermic translocation carriers, a study by Oliver-Bonet et al. (2005a) on a normozoospermic t(10;14) carrier showed a low frequency of asynapsis around the breakpoints. A few sperm were found in the first biopsy of the t(8;13) carrier examined in this study, suggesting that, as long as some spermatocytes contain completely synapsed quadrivalents, a few cells may be able to complete meiosis and produce sperm.

The association between the rearranged chromosomes and the XY body has led to the hypothesis that MSCI may spread to the quadrivalent, leading to the inactivation of autosomal genes (Jaafar et al., 1993). However, recent studies have shown that meiotic silencing may not be limited to the sex chromosomes. Rather, MSCI appears to be an example of a more generalized mechanism known as meiotic silencing of unsynapsed chromatin (MSUC) that silences any asynapsed regions during meiosis, regardless of whether they are on autosomal or sex chromosomes (Turner et al., 2006). In mice, these asynapsed regions are characterized by the localization of BRCA1 along unsynapsed axes, H2AX phosphorylation and transcriptional silencing (Turner et al., 2005). These observations are consistent with our findings in the t(8;13) carrier. Asynapsed regions of the quadrivalents display BRCA1 and H2AX localization, regardless of whether or not they pair with the sex chromosomes. Conversely, quadrivalents displaying heterosynapsis and complete pairing display no clear BRCA1 or H2AX labeling. These observations were consistent with those of Sciurano et al. (2007), who recently found that the asynapsed regions of quadrivalents show both BRCA1 and H2AX localization. Immunostaining for RNA polymerase II provided further evidence that pairing with the sex chromosomes is not required for unsynapsed regions to be transcriptionally silenced. Thus, the hypothesis that MSCI spreads to the quadrivalent is not entirely correct. Although asynapsed quadrivalents associated with the sex chromosomes are silenced, pairing with the sex chromosomes does not appear to be required for this inactivation. These observations support suggestions that asynapsis is the driving force behind meiotic inactivation in humans, and is not specific to the sex chromosomes (Turner et al., 2006). Homolka et al. (2007) recently found that pachytenes spermatocytes of mice who carried a t(16;17) translocation showed a partial suppression of genes near the breakpoint on chromosome 17, providing evidence of MSUC at the mRNA level. Surprisingly, they also found an overexpression of genes on the X chromosome, suggesting that the XY-association of the quadrivalent may also contribute to male sterility by disrupting MSCI (Homolka et al., 2007).
synapsis is a critical factor in determining spermatogenic outcome. In this report, we have provided evidence that unpaired regions, regardless of whether or not they pair with the sex chromosomes, are transcriptionally silenced during human meiosis. If genes critical for meiosis are present in the asynapsed regions, meiotic arrest is likely to occur. Both the chromosomes involved in the translocation and the location of the breakpoints are likely the determining factors for the fidelity of synapsis, and thus the fertility status of the carrier.

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