Meiotic studies in an azoospermic human translocation (Y;1) carrier

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A reciprocal translocation between the long arm of the Y chromosome and the long arm of chromosome 1 was observed in an infertile man with non-obstructive azoospermia. The study was performed using a combination of techniques: immunocyto genetic analysis, which allows the detection of synaptonemal complexes (SCs) and recombination sites (MLH1) simultaneously, and fluorescence in-situ hybridization analysis. Meiotic pairing analysis on 100 pachytene spreads showed the presence of a quadrivalent containing chromosomes 1 and Y. There were many abnormalities in chromosome pairing and recombination. These abnormalities included a great reduction of recombination events (as many as one fifth of the SCs had no MLH1 foci), and high proportions of unpaired regions and discontinuities in the SCs. We discuss the possibility that infertility in this patient may be due to transcriptional repression of part of chromosome 1 involved in the translocation, silencing some genes necessary for the progression of meiosis and causing defective meiotic pairing and recombination.

Key words: immunocyto genetics/meiosis/meiotic recombination/sex-autosome translocation/synaptic anomalies

Introduction

The incidence of Y/autosome translocation (whether balanced or unbalanced) is low (Nielsen and Rasmussen, 1976; Powell, 1984). Translocations of the distal heterochromatic part of the Y chromosome onto the short arm of acrocentric chromosomes usually have no phenotypic effect on the carrier, and fertility is usually unaffected (Cohen et al., 1981; Alitalo et al., 1988; Hsu, 1994). In contrast, the rare translocations of the euchromatic part of the Y chromosome onto non-acrocentric autosomal regions are frequently associated with azoospermia (Hsu, 1994; Schinzel, 2001).

Few cases of reciprocal (Y;1) translocations have been reported (Hsu, 1994; Conte et al., 1996). Most of these studies focused on the description of translocation breakpoints, but provided little insight into the underlying relationship between the Y-autosome translocation and meiotic synopsis. Recently, techniques for fluorescence antibody localization on surface-spread prepared spermatoocytes have been developed and a number of important proteins involved in meiotic synopsis, recombination and cell cycle control have been identified (Ashley and Plug, 1998). Also this analysis allows the various stages of prophase to be identified (Baker et al., 1996; Sun et al., 2004a). This approach opens new avenues for investigations into meiosis.

Here, we present the first report of the meiotic behaviour of a (Y;1) translocation in a man with non-obstructive azoospermia, using a fluorescence immunocyto genetic approach to detect synaptonemal complex (SC) elements and MLH1 foci.

Materials and methods

Patient report

The infertile patient, aged 41 years, was recruited from the University of California San Francisco, San Francisco, USA. His phenotype and clinical examinations were normal, including testicular size (20 ml) and consistency. Hormone evaluation showed normal levels of testosterone, prolactin, FSH and LH. Two semen analyses with centrifuged pellet analyses indicated azoospermia. The patient had one brother and two sisters; both sisters had children. The patient had non-consanguineous parents and no history of cancer in his family. The patient underwent a testicular biopsy and fine needle aspiration mapping (Turek et al., 2000) to determine candidacy for ICSI, but refused a lymphocyte karyotype analysis.

Testicular biopsy

Both cytological and histological examination showed a bilateral, global early maturation arrest pattern. No sperm were detected on fine needle aspiration mapping. Part of the testicular material was kept in phosphate-buffered saline and transferred on ice by air courier to the laboratory in Calgary, Canada, for fluorescence immunocyto genetic analysis. We have previously demonstrated that cold storage of testicular tissue does not affect chromosome synopsis and recombination (Sun et al., 2004c). This study received ethical approval from the institutional review boards of the University of California San Francisco and the University of Calgary.

Fluorescence immunostaining and fluorescence in-situ hybridization

Slides with chromosome spreads were subjected to immunofluorescence staining as described previously (Barlow and Hultén, 1998; Sun et al.,
2004b). Primary antibodies against the following proteins were used: synaptonemal complex protein 1 (SYN1, marks transverse elements of the SC; a gift from P. Moens, York University), synaptonemal complex protein 3 (SCP3, marks lateral elements of the SC; a gift from T. Ashley, Yale University), a mismatch repair protein (MLH1, marks meiotic recombination sites; Oncogene, San Diego, CA, USA) and CREST (Calcinosis, Raynaud’s phenomenon, Esophageal dysfunction, Sclerodactyly, Telangiectasia, marks the centromere; a gift from M. Fritzler, University of Calgary) (Lynn et al., 2002; Sun et al., 2004b). These primary antibodies were detected using a cocktail of secondary antibodies (donkey antisera) conjugated with different fluorochromes [AMCA and Cy3 (Jackson Immunoresearch, West Grove, PA, USA), Alexa 488 and Alexa 555 (Molecular Probes, Eugene, OR, USA)] and examined on a Zeiss Axiophot epifluorescence microscope. Images were captured using Applied Imaging Cytovision 3.1 software (Applied Imaging Corporation, Santa Clara, CA, USA). One hundred pachytene-stage cells were analysed, and the number of MLH1 foci per autosomal bivalent and per cell was scored.

After SC analysis was complete, fluorescence in-situ hybridization (FISH) analysis using a microwave technique (Ko et al., 2001) was carried out on the same cells using centromeric probes for chromosome X, Y, 1 and a sub-telomere-specific probe for chromosome 1p. An X-specific α-satellite probe, kindly provided by E. Jabs of the Johns Hopkins University, Baltimore, MD (Jabs et al., 1989), and a chromosome 1-specific satellite III sequence, pUC1.77, generously provided by H.J. Cooke of Edinburgh, Scotland (Cooke and Hindley, 1979) were labelled directly with Fluorogreen™ and Fluoroblue™ (Amersham, Baie d’Urfe, QC, Canada) by nick translation, respectively. CEP SpectrumOrange Yq probe was purchased from Vysis (Downer’s Grove, IL). A chromosome 1 midisatellite probe labelled with Fluorogreen™ (which hybridizes near the telomere of the short arm of chromosome 1) was purchased from Oncor (Gaithersburg, MD, USA). FISH signals were captured and analysed in the same cells in which SCs had been previously analysed, using the same image system.

Results

Stages of meiotic prophase were distinguished by the appearance and chronology of SC proteins, using immunofluorescent techniques (Sun et al., 2004a). The cells were observed in different stages of prophase I in the following frequencies: 33% in leptotene, 48% in zygotene and only 19% in pachytene, compared to 7, 4 and 88% in controls (Gonsalves et al., 2004). We analysed a total of 100 fluorescence-stained pachytene nuclei, which were recognized by the presence of the sex body. The observed meiotic configurations were classified into different pachytene stages, according to Solari’s XY type classification (Solari, 1980). The analysis of the SC showed the presence of a quadrivalent in all cells (Figure 1A,C,D). FISH analysis of the spreads revealed a reciprocal translocation between the long arm of the Y chromosome and the long arm of chromosome 1 (Figure 1B). Approximately half of the analysed cells presented an open quadrivalent structure, with the axial elements of the sex

Figure 1. Fluorescence micrographs showing meiotic configurations visualized by synaptonemal complexes (SCs) at the pachytene stage (A,C and D). The cells were treated with antibodies against SCP3/SYN1 (to visualize SCs; red), MLH1 (to identify meiotic exchanges; green) and with CREST antiserum (to detect centromeric regions; blue). (A) shows an open quadrivalent configuration at early pachytene: one SC has a split (sp) and three SCs have no MLH1 foci. FISH analysis on the same spermatocytes as in (A) is shown in (B). Chromosome-specific probes were used to identify the centromere of chromosome X (green) and of chromosome 1 (blue), sub-telomere 1p (green) and Yq12 (red). (C) shows the quadrivalent starting to intermingle with the condensed sex body (sb) at late pachytene. (D) is a late pachytene spread with 1q completely intermingled with, and indistinguishable from the sex body (arrow).
chromosomes apparent and no disruption of the pairing between Yp and the pseudoautosomal region of Xp (Figure 1A). The rest of the cells presented the quadrivalent intermingled with the sex body at various stages of condensation (Figure 1C,D), sometimes causing difficulties in discerning which arms belonged to chromosome 1 and which belonged to the sex body (Figure 1D). In addition, a terminal asynapsis in chromosome 1 was found in nearly all analysed cells.

Many meiotic abnormalities in chromosome pairing and recombination were also detected (Table I). The proportion of pachytenes with unpaired chromosome regions (split-like structures in the SC) and with discontinuities (gap-like structures in the SC) was higher than that in normal controls (previously reported by our group) (Sun et al., 2005)(t-test, P < 0.001). The number of MLH1 foci, marking meiotic recombination sites, was greatly reduced with a mean of 34.9 per cell compared to 48.0 per cell in control donors (Sun et al., 2005) (t-test, P < 0.001). In normal controls, the frequency of bivalents with no MLH1 foci was 0.3%, so non-exchange or future aachiasmate chromosome pairs are rare in normal males (Sun et al., 2005). In this case, 469 of 2200 SCs (21%) were determined to be lacking an MLH1 focus (Table I).

### Discussion

This is the first report of immunofluorescence meiotic analysis on a carrier of a t(Y;1)(q:?;q?). In this patient, fluorescent antibody localization detected a quadrivalent structure in meiotic pachytene stages, and characterized dramatic abnormalities in chromosome pairing and recombination.

Conventional cytogenetic and FISH analyses on mitotic chromosomes allow quick and accurate assignment of the breakpoint location, but cannot explain the infertility of some translocation carriers. Meiotic investigations are therefore required. Immunocyto-genetic analysis has been shown to be a useful technique for the detection of synaptic anomalies (Gonsalves et al., 2004; Sun et al., 2004a,b). In this patient, several abnormalities indicating defects in chromosome pairing and recombination were found. The presence of a high proportion of cells in pachyneme suggests a failure to progress through meiosis due to a problem in homologous pairing. Cells that did reach the pachytene stage showed a higher percentage of unpaired chromosome regions and many more discontinuities in the SCs than controls. Meiotic recombination (crossing over) is essential for the segregation of homologous chromosomes and formation of normal haploid gametes. Bivalents with no recombination foci, i.e. future aachiasmates, are unable to orient properly on the metaphase plate or to segregate chromosomes to daughter cells. In the translocation carrier, the number of recombination (MLH1) foci/cell was significantly reduced when compared to normal control values, and approximately one fifth of bivalents did not contain a recombination focus. Thus recombination was also affected in chromosomes not involved in the translocation.

During the first meiotic prophase of mammalian spermatogenesis, sex chromosomes exhibit a characteristic behaviour that includes heterochromatinization (condensation) of the sex body, delayed chromatin replication and transcriptional inactivity (Lifschytz and Lindsley, 1972; Saussine et al., 1994). The hypothesis of the reversal of sex transcriptional inactivation as a response to the attachment of an autosomal chromosome to the sex body was first proposed by Lifschytz and Lindsley (1972). The authors suggested that meiotic sex chromosome inactivation might be critical in order to silence genes on the X chromosome which otherwise would be deleterious to spermatogenesis. An alternative theory proposed that there is a spreading of the sex body inactivation to the translocated autosome, and that inactivation of certain regions in the genome could lead to the arrest of meiosis (Jaafar et al., 1989).

Since there is a strong relationship between the heterochromatinization of the sex body and transcriptional inactivation (Fernandez-Capetillo et al., 2003), it seems unlikely that a reactivation of sex chromosome genes occurred in our patient, as the sex body was observed at different stages of condensation. Moreover, inclusion of the asynapsed segments of chromosome 1 in the sex body exhibited a hypercondensed appearance similar to that of the sex body (Figure 1D). This same behaviour has been observed in a Y;6 translocation carrier (Delobel et al., 1998). Meiotic sex body inactivation is triggered by the phosphorylation of the histone H2AX by the kinase ATR, which is BRCA1 dependent (Turner et al., 2004). In male mice carriers of a T(X;16) translocation, accumulation of ATR has been observed in asynapsed regions of chromosome 16. Asynapsed regions have been shown to be silenced in mice (Turner et al., 2005). The low frequency of recombination events and the delay in the completion of SC formation observed in this translocation carrier could then be associated with the transcriptional repression of the asynapsed regions of chromosome 1, through the BRCA1/ATR-mediated meiotic inactivation of unpaired axes, which would result in the hypercondensation observed and the silencing of some of the genes within this segment which code for proteins involved in meiotic processes, such as *exo1* (located on 1q42–q43) (Genschel et al., 2002). The presence of this general disturbance in recombination and synapsis may trigger a pachytene checkpoint (Plug et al., 1997, 1998), leading to maturation arrest of the primary spermatocytes and consequently contributing to the azoospermia found in this translocation carrier.

In addition, the presence in this carrier of a subset of cells arrested at zygote may suggest the presence of at least two different checkpoints working at different times, one checkpoint acting before the onset of pachytene and the other acting during pachytene. We have observed a partial zygote block with pachytene abnormalities in one other azoospermic patient (Sun et al., 2004a). Thus, spermatocytes that fail to complete synopsis before the activation of any of these checkpoints may be caught and pushed into an apoptotic pathway (Odorisio et al., 1998), resulting in sterility (Ashley and Plug, 1998; Ashley, 2002).

### Table I. Frequency of autosomal SCs with zero to five MLH1 foci, and fidelity of chromosome pairing in a (Y;1) translocation patient and normal controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean no. autosomal SCs when no. of foci is</th>
<th>MLH1 foci</th>
<th>No. gaps</th>
<th>Mean no. ± SD</th>
<th>Range</th>
<th>% Cells with gaps</th>
<th>No. splits</th>
<th>% Cells with splits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1</td>
<td>3.5</td>
<td>12.3</td>
<td>4.9</td>
<td>1.1</td>
<td>0.2</td>
<td>48.0 ± 4.7</td>
<td>21 – 65</td>
</tr>
<tr>
<td>t(Y;1)</td>
<td>4.7</td>
<td>5.5</td>
<td>7.4</td>
<td>3.2</td>
<td>3.2</td>
<td>0.2</td>
<td>34.9 ± 18.4</td>
<td>2 – 64</td>
</tr>
</tbody>
</table>

*a1100 and 100 pachytene stage cells were analysed in controls and the patient, respectively.

*bGap refers to a discontinuity in the SC.

*cSplit refers to an unpaired region of the SC.
In summary, we propose that the synapsis and heterocromatization observed in this translocation carrier are responsible for gene inactivation over some loci on the long arm of chromosome 1. It is then this silencing of critical autosomal genes that contributes to the events that lead to the azoospermia observed in this patient, rather than the reactivation of genes located on the sex chromosomes. Furthermore, our results add to the evidence that operation of different checkpoints detects asynapsis and abnormal recombination during meiotic prophase, which then results in meiotic arrest and cell degeneration (Odorisio et al., 1998).

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References


Cooke HJ and Hindley J (1979) Cloning of human satellite III DNA: differences in inactivation over some loci on the long arm of chromosome 1. It is then this silencing of critical autosomal genes that contributes to the events that lead to the azoospermia observed in this patient, rather than the reactivation of genes located on the sex chromosomes. Furthermore, our results add to the evidence that operation of different checkpoints detects asynapsis and abnormal recombination during meiotic prophase, which then results in meiotic arrest and cell degeneration (Odorisio et al., 1998).


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