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ARTICLE

Abnormal meiotic recombination with complex chromosomal rearrangement in an azoospermic man

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Abstract Spermatocyte spreading and immunostaining were applied to detect meiotic prophase I progression, homologous chromosome pairing, synapsis and recombination in an azoospermic reciprocal translocation 46, XY, t(5;7;9;13)(5q11;7p11;7p15;9q12;13p12) carrier. Histological examination of the haematoxylin and eosin stained testicular sections revealed reduced germ cells with no spermatids or sperm in the patient. TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling assay showed apoptotic cells in testicular sections of translocation carrier. Immnunofluorescence analysis indicated the presence of an octavalent in all the pachytene spermatocytes analysed in the patient. Meiotic progression was disturbed, as an increase in zygotene ($P < 0.001$) and decrease in the pachytene spermatocytes ($P < 0.001$) were observed in the t(5;7;9;13) carrier compared with controls. It was further observed that 93% of octavalents were found partially asynapsed between homologous chromosomes. A significant decrease in the recombination frequency was observed on 5p, 5q, 7q, 9p and 13q in the translocation carrier compared with the reported controls. A significant reduction in XY recombination frequency was also found in the participants. Our results indicated that complex chromosomal rearrangements can impair synaptic integrity of translocated chromosomes, which may reduce chromosomal recombination on translocated as well as non-translocated chromosomes, a phenomenon commonly known as interchromosomal effect.

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Introduction

Complex chromosomal rearrangements (CCR) are constitutive structural aberrations involving three or more chromosomal breaks accompanied by exchange of genetic information (Pellestor et al., 2011). They are rare events in humans, and only 250 cases of CCR have been reported worldwide to date (Madan, 2012). More extensive studies are required to understand the underlying mechanisms of CCR leading to male infertility. Most men with CCR suffer from infertility-associated problems caused by spermatogenic failure and hypospermatogenesis (Chandley, 1981; Coco et al., 2004). Female carriers of CCR are at risk of conception with various anomalies and of reproductive failure, owing to abnormal segregation of the derivative chromosome or meiotic failure (Kuechler et al., 2005). Shi and Martin (2001) reported that chromosomal translocation may interfere with the segregation of other chromosomes, an interchromosomal effect (ICE) that increases the risk of unbalanced sperm that can lead to problematic conception. Kirkpatrick and Ma (2012), however, reported that a conception from these patients would lead to a severe risk of spontaneous abortion or neonatal mental retardation.

It has previously been reported that azoospermic carriers with reciprocal translocations have a high degree of asynapsis around the breakpoints. Four chromosomes were involved in several of such reciprocal translocations, and these chromosomes displayed a cross-shaped structure known as a quadrivalent during the first meiotic division with different degrees of asynapsis around the break points (Chandley et al., 1986; Jiang et al., 2014; Leng et al., 2009). Among the CCR, the most common type observed during meiosis I is hexavalent configuration with efficient synapsis between fully paired six chromosomal arms except in the small central segments around the breakpoints (Johannisson et al., 1988; Kovacs et al., 1992; Saadallah and Hulten, 1985).

To shed light on possible aspects of CCR causing human male infertility, synapsis and recombination of homologous chromosomes are reported in a patient with CCR using fluorescence immunocytogenetic approach.

Materials and methods

Patient and karyotype analysis

A 45-year old man presented to the Fourth Affiliated Hospital of Anhui Medical University, Hefei, Anhui, People's Republic of China. Semen analysis was carried out in accordance with the World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-ervical Interaction (2010). The analysis revealed that the patient had azoospermia. After obtaining written informed consent from the patient, testicular tissues were sampled. Five fertile men, having at least one healthy child, were recruited as normal controls for this study, and similar tests were conducted on them. All the experimental procedures and protocols were approved by the Institutional Review Board at the University of Science and Technology of China on 1 April 2014; the approval ID is USTCEC201400003.

Spermatocyte spreads and immunostaining

Testicular tissues were processed as described previously (Jiang et al., 2014; Pan et al., 2012). Rabbit anti-SYCP3 (donated by Christa Heyting, Wageningen University, Netherlands), human anti-CREST (Immunovision, Springdale, AR), mouse anti-MLH1 (BD Pharmingen Biosciences, San Diego, CA), mouse anti-Brca1 (Santa Cruz Biotechnology, Santa Cruz, California, USA) and mouse anti-γ-H2AX (Millipore, Billerica, MA) were used as primary antibodies. The primary antibodies were detected using the following secondary antibodies: Alexa 555 donkey anti-rabbit (Molecular Probes, Carlsbad, California, USA), Alexa 488 goat anti-mouse (Molecular Probes, Carlsbad, California, USA), Alexa 488 donkey anti-mouse (Molecular Probes, Carlsbad, California, USA) and 1-amino-4-methylcoumarin-3-acetic acid (AMCA) donkey anti-human (Jackson Immunoresearch, West Grove, Pennsylvania, USA).

Fluorescence in-situ hybridization

The spermatocyte spreads previously immunostained for meiotic analyses were used for fluorescence in-situ hybridization using centromeric probes to identify the translocated chromosomes. The CEP5 and CEP9 probes were labelled with Spectrum Green dUTP (Vysis, 02N32-050), whereas the CEP7 and the CEP13 probes were labelled with Spectrum Orange dUTP (Abbott, 02N33-050) using a nick translation procedure following the manufacturer's instructions.

TUNEL assay

Testicular tissues for haematoxylin and eosin (H&E) staining were fixed over night in 4% paraformaldehyde at room temperature followed by embedding in paraffin. A TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) assay was used to detect apoptotic cells by using In Situ Cell Death Detection Kit (Roche, Mannheim, Germany).

An epifluorescence microscope Olympus BX61 (Olympus Inc., Tokyo, Japan) and Image Pro-Plus version 5.1 software (Media Cybernetics Inc., Bethesda, Maryland, USA) were used for imaging and cell evaluation.

Statistical analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 13.0 (SPSS Inc., Chicago, IL, USA). A chi-squared test was applied to compare rates of recombination in XY bivalents and meiotic