Reproductive BioMedicine Online (2012) 24, 91–100

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Meiotic recombination, synapsis, meiotic inactivation and sperm aneuploidy in a chromosome 1 inversion carrier

Gordon Kirkpatrick, Victor Chow, Sai Ma *

Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada V6H-3N1
* Corresponding author. *E-mail address:* sai@interchange.ubc.ca (S Ma).

Gordon Kirkpatrick is currently a second-year medical student at the University of British Columbia (UBC). He graduated from UBC with an MSc. in Reproductive and Developmental Sciences. Under the supervision of Dr. Sai Ma, Gordon examined meiotic behavior in germ cells form infertile men with and without chromosome abnormalities.

Abstract Disrupted meiotic behaviour of inversion carriers may be responsible for suboptimal sperm parameters in these carriers. This study investigated meiotic recombination, synapsis, transcriptional silencing and chromosome segregation effects in a pericentric inv(1) carrier. Recombination (MLH1), synapsis (SYCP1, SYCP3) and transcriptional inactivation (γH2AX, BRCA1) were examined by fluorescence immunostaining. Chromosome specific rates of recombination were determined by fluorescence in-situ hybridization. Furthermore, testicular sperm was examined for aneuploidy and segregation of the inv(1). Our findings showed that global recombination rates were similar to controls. Recombination on the inv(1) and the sex chromosomes were reduced. The inv(1) associated with the XY body in 43.4% of cells, in which XY recombination was disproportionately absent, and 94.3% of cells displayed asynapsed regions which displayed meiotic silencing regardless of their association with the XY body. Furthermore, a low frequency of chromosomal imbalance was observed in spermatozoa (3.4%). Our results suggest that certain inversion carriers may display unimpaired global recombination and impaired recombination on the involved and the sex chromosomes during meiosis. Asynapsis or inversion-loop formation in the inverted region may be responsible for impaired spermatogenesis and may prevent sperm-chromosome imbalance.

KEYWORDS: chromosomal rearrangements, infertility, meiosis, meiotic silencing of unsynapsed chromatin, recombination

Introduction

In order to pair during meiosis, inversions theoretically adopt an inversion loop to allow the homologous partner to synapse. Despite improved synapsis between the inverted and normal chromosome, asynapsis does occur. Homosynapsis of the distal regions with either asynapsis or heterosynapsis of the inverted region has been observed (Gabriel-Robez and Rumpler, 1994).

Disrupted meiotic behaviour of inversion carriers may be responsible for abnormal sperm parameters in these carriers. The formation of the inversion loop could disrupt

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2011.09.013

other meiotic machinery and impede progression through meiosis (Forejt et al., 1981). In addition, reduced recombination has been observed within the inversion loop, which in general has been shown to have a detrimental effect on meiosis (Brown et al., 1998).

Even in cases of heterosynapsis of the inverted region, inversion carriers are at risk for producing chromosomally abnormal spermatozoa. If an odd number of crossovers occurs within the inverted sequence, then resulting spermatozoa will have an unbalanced chromosome complement. The frequency of unbalanced spermatozoa in inversion carriers has been studied in numerous carriers (reviewed in Anton et al., 2005) and has been shown to be as low as 0%, when the segment is very short (Balkan et al., 1983), and as high as 30% when the segment is long (Navarro et al., 1993). Variation in aneuploidy levels likely reflects the variation in the chromosome involved, the proportion of the chromosome involved in the inversion and the likelihood of a recombination event occurring within the inverted region. Furthermore, as the frequency of unbalanced spermatozoa reflects the proportion of unbalanced embryos, analysis of inversion segregation would allow for a more personalized risk assessment for unbalanced offspring in inversion carriers.

During the first meiotic division, chromosome synapsis is facilitated by the synaptonemal complex. Immunofluorescence techniques have allowed the detailed study of pairing and recombination and have provided insight into the role of meiotic errors in infertility. Infertile men display reduced recombination and increased synaptic errors (Ferguson et al., 2007; Gonsalves et al., 2004; Judis et al., 2004; Ma et al., 2006; Sun et al., 2004, 2008). Meiotic defects may be caught by meiotic checkpoints, leading to spermatogenic arrest and reduced sperm concentration (Baker et al., 1995; Edelmann et al., 1996). Furthermore, errors in both the frequency (Hassold et al., 1991; Reish et al., 2004; Shi et al., 2001) and distribution (Ferguson et al., 2009; Hassold et al., 1995; Lamb et al., 1996) of crossovers have been linked with non-disjunction and the production of aneuploid spermatozoa.

Immunofluorescence studies of carriers of chromosome abnormalities such as inversions have been limited. Six meiotic studies in human males have been carried out in translocations carriers (Ferguson et al., 2008; Leng et al., 2009; Oliver-Bonet et al., 2005; Pigozzi et al., 2005; Sciurano et al., 2007; Sun et al., 2005) in which quadrivalents displayed a high frequency of asynapsis and a tendency to associate with the sex chromosomes. Electron microscopy studies have also indicated a preferential association of chromosome abnormalities with the sex chromosomes in reciprocal translocations (Chandley et al., 1986; Luciani et al., 1987), Robertsonian translocations (Luciani et al., 1984; Rosenmann et al., 1985) and numerical abnormalities (Johannisson et al., 1983).

During meiosis, the X and Y chromosomes pair along two small pseudoautosomal regions and the XY body undergoes transcriptional silencing, known as meiotic sex-chromosome inactivation (MSCI; Handel, 2004; Solari, 1974). MSCI involves localization of phosphorylated H2AX (Celeste et al., 2002) and BRCA1 (Turner et al., 2004; Xu and Stern, 2003). XY body association has been noted in chromosome abnormalities with a high degree of asynapsis (Chandley et al., 1986; Ferguson et al., 2008) and may allow silencing of the unsynapsed regions of the rearrangement in a similar manner to MSCI. Chromosome abnormalities displaying XY body association have shown reduced transcription in the chromosome abnormalities and increased transcriptional activity on the X chromosome (Homolka et al., 2007). This has led to concern that XY body association can result in disruption of XY body inactivation leading to activation of meiotic checkpoints, meiotic arrest and reduced sperm parameters.

As far as is known, this is the first immunofluorescent examination of recombination and synapsis in an inversion (inv(1)) carrier. This study combined fluorescent in-situ hybridization (FISH) with immunofluorescent techniques to study synapsis and recombination, on the inverted chromosome as well as chromosomes 13, 18, 21 and the sex chromosomes. This also allowed the presence of XY body association to be determined. FISH on spermatozoa was used to determine the relationship between recombination and aneuploidy in specific chromosomes. Finally, using antibodies for γH2AX and BRCA1, this study assessed the transcriptional inactivation of the unsynapsed regions of the inversion.

Materials and methods

Patient ascertainment

A 45-year-old male was presented with a 3-year history of infertility. He displayed azoospermia, normal hormonal profiles and absence of any physical obstruction in the reproductive tract. However, a karyotype showed that he is a heterozygous carrier of inv(1) (p21q31; **Figure 1**). Subsequently, he underwent a testicular biopsy to extract spermatozoa for intracytoplasmic sperm injection (ICSI); however, insufficient spermatozoa were retrieved to perform ICSI. A histological examination found germ-cell-maturation arrest. A small portion of tissue was used for meiotic analyses in the present study. Five control tissue samples were retrieved from proven fertile men with normal karyotypes, who were undergoing vasectomy reversals. Informed consent was sought prior to surgery for all participants in this study. Institutional Review Board approval was obtained from the University of British Columbia Research Ethics Boards (H06-70325).

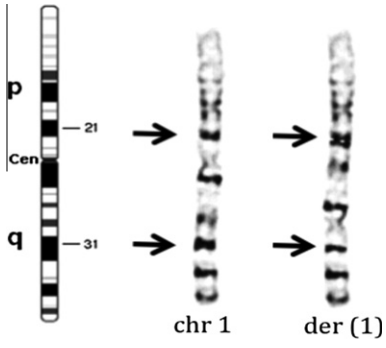

Figure 1 Karyotype of chromosome 1 and the inverted der(1) chromosome. Idiogram and karyotypes of chromosome 1 and the inverted chromosome, with arrows at the location of the breakpoints at p21 and q31.