Meiotic non-disjunction mechanisms in human fertile males

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BACKGROUND: In humans, little is known about the mechanisms of non-disjunction working in male meiosis, although considerable attention has been given to these mechanisms in female meiosis. The present study explores the origin of meiotic non-disjunction during human spermatogenesis and the chromosomes most commonly involved in this process.

METHODS: We used Multiplex fluorescence in situ hybridization to carry out meiotic analyses in metaphase I (MI) and metaphase II (MII) spermatocytes from three fertile donors. Testicular biopsy was obtained during a vasectomy procedure.

RESULTS: We examined a total of 317 MI and 248 MII spermatocytes. The frequency of numerical chromosome abnormalities at MII (14.5%) was 5.5 times higher than at MI (2.5%). We observed 88 (27.7%) spermatocytes I with chromosome bivalents with a low chiasma count, usually small chromosomes displaying two separated univalents. Chromosomes X, Y and 21 were the most commonly found as achiasmate chromosomes at MI and the most frequently involved in disomy at MII. Hyperploidy frequency in spermatocytes II (disomy) was significantly higher (P < 0.001) than that found in spermatocytes I (trisomy).

CONCLUSIONS: Achiasmate non-disjunction and premature separation of sister chromatids appear to be the two main non-disjunction mechanisms during the first meiotic division in human spermatogenesis, and both mechanisms contribute equally to the genesis of aneuploidy. The elevated frequencies of disomy detected in spermatocytes II are significantly higher than those previously described in human spermatozoa, suggesting the existence of a postmeiotic checkpoint monitoring numerical abnormalities.

Key words: chromosome abnormalities / meiosis / meiotic segregation / non-disjunction / spermatogenesis

Introduction

In humans, trisomy is the most common chromosome abnormality and it is the leading cause of miscarriage and mental retardation. Around 90% of trisomies originate during paternal or maternal meiosis (Hassold et al., 2007). Paternal origin of autosomal trisomies is low (0–30% of cases, depending on the chromosome affected) (reviewed by Hall et al., 2007), whereas the paternal contribution to sex chromosome trisomies is more clinically relevant (10% in 47,XXX conditions, 50% in 47,XXY cases and 100% in 47,XYY individuals) (Hall et al., 2006). Trisomy is the result of chromosome mis-segregation, basically through meiotic non-disjunction (Márquez et al., 1996). Three main non-disjunction mechanisms have been proposed on the basis of data obtained from human oocytes II (Hassold and Hunt, 2001). First, the failure to resolve chiasmata between homologous chromosomes at anaphase I, known as ‘true’ non-disjunction. Second, the achiasmate non-disjunction, characterized by the absence of chiasmata between a pair of homologs followed by their migration to the same pole of the meiotic spindle. The third mechanism, called premature separation of sister chromatids (PSSC), consists of chromatid segregation during anaphase instead of whole chromosome segregation (Angell, 1991). Direct studies on metaphase II (MII) oocytes report achiasmate non-disjunction and PSSC as the main mechanisms generating aneuploidy in female meiosis I (reviewed by Pellestor et al., 2005, 2006; Rosenbusch, 2006). However, little is known about the relevance of each individual non-disjunction mechanism in human male meiosis I and its contribution to the production of aneuploid spermatozoa.

Basically, non-disjunction in human male meiosis I may result either from improper pairing, abnormal synopsis or anomalous recombination during prophase I, or from abnormal chromosome orientation and premature loss of cohesion between chromatids at anaphase I. Studies in trisomic conceptuses (reviewed by Lamb et al., 2005) and in pachytene spermatocytes and spermatozoa from infertile men (Ferguson et al., 2007; Sun et al., 2008) found an association between abnormal meiotic recombination and aneuploidy. However, these previous works were not able to determine the causative mechanisms of meiotic non-disjunction because the analysis of such mechanisms...
requires the combined cytogenetic analysis of spermatocytes at metaphase I (MI) and MI. Studies at these meiotic stages are scarce, owing to the difficulty of obtaining testicular tissue. Meiotic analyses carried out on fertile and infertile men described abnormal chiasma count and numerical chromosome abnormalities (Skakkebaek et al., 1973; Chandley et al., 1976; Koulischer et al., 1982; Egozcue et al., 1983; Laurie et al., 1985; Guichaoua et al., 1986; Uroz et al., 2009, 2011) but they were not focused on studying non-disjunction mechanisms.

Multiplex fluorescence in situ hybridization (M-FISH), first described in somatic cells (Speicher et al., 1996), allows the simultaneous identification of each human chromosome with a specific combination of five fluorochromes. This technique has been recently applied to meiotic spermatocytes in order to identify meiotic abnormalities (Sarrate et al., 2004) or to analyze non-disjunction in one infertile man (Uroz et al., 2008).

The aims of the present work are (a) to detect the mechanisms causing non-disjunction in human male meiosis I and (b) to identify the chromosomes most commonly involved in aneuploid spermatocytes. We have analyzed spermatocytes at MI and MII stages from three fertile men by M-FISH.

Materials and Methods

Subjects

Testicular samples were collected during a vasectomy procedure from three healthy fertile males, aged 28, 36 and 41 years. Spermatocyte metaphases I from all three donors were previously analyzed by uniform stain in a larger study (Uroz et al., 2011) (donors 16, 8 and 13, respectively), and they were selected on the basis of the number and quality of their meiotic cells. All three subjects produced at least one naturally conceived offspring. One of the donors was a smoker (case 1) and none of them had been exposed to known mutagens or radiation. Semen samples from the individuals analyzed in our study were not available. All donors signed an informed consent form prior to the surgery and the study was approved by our University's and Institutional Ethics Committees.

Spermatocyte spreading

Testicular biopsy was obtained under local anesthesia during a vasectomy procedure and meiotic cells were fixed according to the protocol previously described by our group (Uroz et al., 2008). Meiotic chromosome preparations were preserved at −20°C until M-FISH hybridization.

The M-FISH technique

The M-FISH technique employs whole-chromosome probes labeled with different combinations of 5 fluorochromes for the simultaneous identification of the 24 human chromosomes (Speicher et al., 1996). Meiotic chromosome spreads were hybridized by M-FISH following the manufacturer’s instructions (SpectraVysion Assay, Vysis Inc., Downers Grove, IL, USA), with minor modifications. Briefly, slides were washed in 2 x standard saline citrate/0.1% NP-40 for 5 min, dehydrated in an ethanol series and air-dried. Meiotic preparations were pretreated with 0.005% pepsin for 5 min at 37°C, post-fixed in 1% formaldehyde for 10 min and denatured in 70% formamide for 1.5 min at 73°C. Meiotic chromosome spreads were later counterstained with 4',6-diamidino-2-phenylindole (DAPI) in antifade solution (DAPI II, Vysis Inc., Downers Grove, IL, USA).

Data collection and meiotic analysis

Spermatocyte images were captured under an epifluorescence microscope (Olympus Bx60, Hamburg, Germany) using a Power Macintosh. The M-FISH technique required the capture of each fluorochrome in separate images and metaphase analysis was carried out in the merged image. MI and MII were analyzed with the SpectraVysion software (Digital Scientific, Cambridge, UK) by two different observers, and chromosome abnormalities were classified according to the International System for Human Cytogenetic Nomenclature (ISCN) (Schaffer et al., 2009). Conservative estimates of aneuploidy were calculated by doubling the hyperploidy frequency to avoid technical artifacts. Hypoploid metaphases were included in this study only when showing other chromosome abnormalities. Only spermatocyte II metaphases with 46 chromosomes distributed randomly in a nucleated metaphase were considered diploid.

Statistical analyses

The two-tailed Fisher’s exact test was performed to determine whether there were significant differences in chromosome abnormalities between MI and MII spermatocytes or whether the frequencies of X- and Y-bearing MI spermatocytes differed from the expected 1:1 ratio. Statistical analyses were performed using the Statistical Package for the Social Sciences, and P-values < 0.05 were considered significant.

Results

A total of 565 spermatocytes I (317) and II (248) from three fertile donors were karyotyped by M-FISH. Synaptic or recombination errors were detected by counting the chiasma number in each bivalent. Both chromosome segregation disorders and premature loss of chromatid cohesion were analyzed in MII spermatocytes.

Meiotic analysis was carried out in metaphases, simultaneously stained with DAPI and hybridized by M-FISH (Fig. 1). DAPI counterstain enabled (a) the detection of meiotic chromosome abnormalities and (b) the differentiation between the p and q arm in most chromosomes by marking centromeres as darker spots. Meanwhile, M-FISH colored images allowed the identification of the chromosomes involved in meiotic chromosome abnormalities.

Chiasma number in MI spermatocytes

In MI, sex chromosomes had one chiasma in 73.5% of cases (linear bivalent configuration) (Fig. 1A and B) and two chiasmata in 1.3% (circular bivalent configuration). The remaining 25.2% of spermatocytes showed dissociated sex chromosomes. We also observed the presence of two univalents for autosomes 10, 13, 21 and 22 in 1.9% of MI cells, and monochiasmate medium-sized bivalents for chromosome pairs 7 and 8 in 0.6% (Fig. 1C and D).

Numerical chromosome abnormalities in MI and MII spermatocytes

The frequencies of numerical chromosome abnormalities in MI and MII spermatocytes are shown in Table I. Hyperploidy frequency in spermatocytes II (disomy) was significantly higher (P < 0.001) than that found in spermatocytes I (trisomy). Four trisomic spermatocytes I (1.3%) contained an extra chromosome (univalent) 18, 19, 22 or Y. Disomy at MII (6.9%) was observed as either an extra chromosome (3.6%) or an extra chromatid (3.2%) (Fig. 1G and H). It involved
essentially sex chromosomes (3.2%) and chromosome 21 (1.2%) (Table I). Diploidy was found in 0.8% of the MII spermatocytes.

Chromosome cohesion abnormalities in MII spermatocytes
Separated sister chromatids at MII were seen in 6.9% of the cells.

Discussion
The use of M-FISH for karyotyping MI and MII spermatocytes from fertile men provides a good approach to study the mechanisms of non-disjunction in human male meiosis I. M-FISH is essentially useful in (a) identifying chromosomes involved in aneuploides, (b) increasing the number of analyzable spermatocytes, especially at the MII stage, where there is a high level of overlapping and touching chromosomes and (c) distinguishing diploidy at MII from two juxtaposed metaphases. Although M-FISH is too expensive and time-consuming to be used in clinical routine screening, it is a powerful technique to identify chromosome abnormalities, and it could be suitable for those cases requiring an accurate meiotic analysis.

Low chiasma count in human spermatocytes I
The main abnormality found in meiosis I is the presence of bivalents with a decreased number or absence of chiasmata. Chromosome 21, the smallest one in the karyotype, and the sex chromosomes, whose pairing is limited to the pseudoautosomal region, appear frequently as two separated univalents. In accordance with our results, other meiotic studies in fertile male donors reported G-group and sex chromosomes as the most susceptible to having no chiasmata at MI (Skakkebaek et al., 1973; Uroz et al., 2011) or no recombination foci at pachytene (Sun et al., 2006) (reviewed by Tempest, 2011). The absence of chiasmata has been correlated with an abnormal chromosome segregation in meiosis I both in trisomic 21 (Savage et al., 1998; Oliver et al., 2009) and 47,XXY conceptuses (Hassold et al., 1991; Lorda-Sanchez et al., 1992; Thomas et al., 2000). In the present work, chromosomes X, Y and 21 were the most commonly implicated in disomy in spermatocytes II, corroborating the results reported in spermatozoa from healthy men using FISH (reviewed by Templado et al., 2005, 2011).

Origin of disomy and diploidy in human spermatocytes
The 5.5-fold increase in the frequency of aneuploidy in spermatocytes II versus spermatocytes I suggests that aneuploidy originates mainly by non-disjunction during anaphase I, and is probably caused by missegregation of bivalents showing no chiasmata or decreased chiasma count (27.7%). The occurrence of trisomy in spermatocytes I (2.5%) implies that some aneuploidy in human spermatozoa originates by pre-existing aneuploidy in the germ cells.

The presence of diploidy at MII and its absence in MI spermatocytes shows that this abnormality arises principally during the first meiotic division. Some authors (reviewed by Egozcue et al., 2000) have suggested that spermatocytes I with univalents generate diploid spermatocytes II, since spermatocytes I bypass the spindle assembly checkpoint without carrying out cytokinesis in meiosis I.

On the other hand, the detection of spermatocytes II with separated sister chromatids provides the first evidence that balanced PSSC (or balanced pre-division), described in fresh oocytes II (Sandalinas et al., 2002; Garcia-Cruz et al., 2010), also occurs in human male meiosis. Separated sister chromatids may be at risk of improper segregation at anaphase II, leading to aneuploid spermatozoa. However, it is not possible to rule out that some of the spermatocytes

Figure 1 Human spermatocytes at metaphase stage. Metaphase spermatocytes were analyzed sequentially by DAPI counterstain (A, C, E, G) and by M-FISH (B, D, F, H). Normal MI (A, B). MI with a bivalent seven showing one single distal chiasma (C, D). Normal MII (E, F). MII with an extra X chromatid (G, H).
with separated sister chromatids could correspond in fact to early ana-
phase II.

Non-disjunction mechanisms in meiosis I
We have detected three different non-disjunction mechanisms in
fertile men leading to aneuploidy during the first meiotic

### Table I  Meiotic chromosome abnormalities in 317 MI and 248 MII spermatocytes from three human fertile donors

<table>
<thead>
<tr>
<th>MI spermatocytes</th>
<th>No. (%)</th>
<th>MI spermatocytes</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal chiasma count</td>
<td>88 (27.8)</td>
<td>Chomatid separation</td>
<td>17 (6.9)</td>
</tr>
<tr>
<td>Sex chromosomes</td>
<td>80 (25.2)</td>
<td>Sex chromosomes</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>24;X,Y (75 cells)</td>
<td></td>
<td>23, −X,+Xcht,+Xcht,+21</td>
<td></td>
</tr>
<tr>
<td>24;X,Y,−I(21),+I(21)</td>
<td></td>
<td>22, −X,+Xcht</td>
<td></td>
</tr>
<tr>
<td>24;X,Y,+ace(1)</td>
<td></td>
<td>21,+Xcht, −Y,+Ycht,+Ycht,−14,+14cht,+14cht</td>
<td></td>
</tr>
<tr>
<td>23;X,Y,−I(6),+ace(10),−I(21),+I(21)</td>
<td></td>
<td>Autosomes</td>
<td>14 (5.7)</td>
</tr>
<tr>
<td>24;X,Y,+ace(22)</td>
<td></td>
<td>21,+Xcht, −Y,+Ycht,+Ycht,−14,+14cht,+14cht</td>
<td></td>
</tr>
<tr>
<td>25;X,Y,+der(7)</td>
<td></td>
<td>22,X, −1,+1cht,+1cht</td>
<td></td>
</tr>
<tr>
<td>Autosomes</td>
<td>8 (2.5)</td>
<td>22,Y, −2,+2cht,+2cht</td>
<td></td>
</tr>
<tr>
<td>23;XY,I(7)(Xma = 1)</td>
<td></td>
<td>22,X, −3,+3cht,+3cht</td>
<td></td>
</tr>
<tr>
<td>23;XY,I(8)(Xma = 1)</td>
<td></td>
<td>22,X, −3,+3cht</td>
<td></td>
</tr>
<tr>
<td>23;XY,−I(10),+I(10)</td>
<td></td>
<td>22,X, −6,+6cht,+6cht</td>
<td></td>
</tr>
<tr>
<td>23;XY,chrb(3),−I(13),+I(13),+I(13)</td>
<td></td>
<td>20,X, −6,+6cht,+6cht,+7cht,+7cht,−7,+13cht,+13cht</td>
<td></td>
</tr>
<tr>
<td>23;XY,−I(21),+I(21)</td>
<td></td>
<td>22,Y, −7,+7cht,+7cht</td>
<td></td>
</tr>
<tr>
<td>24;XY,−I(21),+I(21)</td>
<td></td>
<td>22,Y,chrb(7), −8,+8cht,+8cht,chrb(8)</td>
<td></td>
</tr>
<tr>
<td>23;XY,−I(6),+ace(10),−I(21),+I(21)</td>
<td></td>
<td>22,Y, −13,+13cht,+13cht</td>
<td></td>
</tr>
<tr>
<td>24;XY,−I(22),+I(22),+I(22)</td>
<td></td>
<td>22,Y, −22,+22cht</td>
<td></td>
</tr>
<tr>
<td>Conservative aneuploidy</td>
<td>8 (2.5)</td>
<td>22,Y, +ace(7),−22,+22cht,+22cht</td>
<td></td>
</tr>
<tr>
<td>Numerical abnormalities</td>
<td>4 (1.3)</td>
<td>Numerical abnormalities</td>
<td>36 (14.5)</td>
</tr>
<tr>
<td>Trisomy</td>
<td></td>
<td>Disomy</td>
<td>17 (6.9)</td>
</tr>
<tr>
<td>24;X,I(Y)</td>
<td></td>
<td>Extra whole chromosome</td>
<td>9 (3.6)</td>
</tr>
<tr>
<td>23;XY,−I(4),+I(8)</td>
<td></td>
<td>24;XY (3 cells)</td>
<td></td>
</tr>
<tr>
<td>24;XY,+I(19)</td>
<td></td>
<td>24;XY,chrb(X)(q?)</td>
<td></td>
</tr>
<tr>
<td>24;XY,+I(22)</td>
<td></td>
<td>24;XY, −6,+7,−11,+12</td>
<td></td>
</tr>
<tr>
<td>Conservative aneuploidy</td>
<td>8 (2.5)</td>
<td>23, −X or −Y,+9</td>
<td></td>
</tr>
<tr>
<td>Diploidy</td>
<td></td>
<td>Extra chromatid</td>
<td>8 (3.2)</td>
</tr>
<tr>
<td>23,Y,+Xcht</td>
<td></td>
<td>23,Y,+Xcht</td>
<td></td>
</tr>
<tr>
<td>21,+Xcht, −Y,+Ycht,+Ycht,−14,+14cht,+14cht</td>
<td></td>
<td>23,X,−Xcht</td>
<td></td>
</tr>
<tr>
<td>23;X,+4cht</td>
<td></td>
<td>23,X,−16cht</td>
<td></td>
</tr>
<tr>
<td>23;Y,+15cht</td>
<td></td>
<td>23,X,+21cht</td>
<td></td>
</tr>
<tr>
<td>23;Y,+21cht</td>
<td></td>
<td>23,−Y,+21cht</td>
<td></td>
</tr>
<tr>
<td>Conservative aneuploidy</td>
<td>34 (13.7)</td>
<td>34 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Diploidy</td>
<td>2 (0.8)</td>
<td>46;XY (2 cells)</td>
<td></td>
</tr>
</tbody>
</table>

M-FISH, multiplex fluorescence in situ hybridization.

*Spermatocytes showing more than one meiotic chromosome abnormality were listed in each category.

PSSC, characterized by the presence of an extra (or missing) chromo-
matid at MI (Angell, 1991), was detected in around 50% of disomies
observed in spermatocytes II. This non-disjunction mechanism is
common in human oocytes (Pellestor et al., 2006; Jones, 2008;
Garcia-Cruz et al., 2010), and has been recently described by our
According to our data, chromosomes X, Y and 21 are the most commonly affected by PSSC in MII spermatocytes and correspond to those frequently found as separated univalents at MI. A cytogenetic study of mouse oocytes proposed that extra or missing chromatids in oocytes II arise by bi-orientation of univalents and their mitotic-like segregation during meiosis I (Kouznetsova et al., 2007).

The remaining 50% of disomies found in spermatocytes II had an extra whole chromosome that could originate from achiasmate non-disjunction. This is based on the high incidence both of sex chromosome univalency at MI and of XY disomy at MII. Accordingly, high levels of sex chromosomes with no recombination focus in pachytene spermatocytes have been related to increased XY disomy in spermatozoa (Sun et al., 2008).

The medium-sized monochiasmate bivalents observed in this study, previously described in infertile males (Templado et al., 1981), could be at risk of missegregation. The open configuration of these bivalents may favor an interlocking with other bivalents aligned in the metaphase plate, resulting in non-disjunction during meiosis I (McDermott, 1966). A recent study in yeast has demonstrated that bivalents with a single distal chiasma are prone to mono-orientation (Lacefield and Murray, 2007). This mechanism could probably correspond to ‘true’ non-disjunction. Alternatively, bivalents having a single distal chiasma could undergo a premature chiasma resolution resulting in achiasmate non-disjunction of the two separated homologous chromosomes (McDougall et al., 2005).

Figure 2 Non-disjunction mechanisms in human male meiosis I producing aneuploid spermatozoa. (A) Normal segregation of homologous chromosomes during meiosis I. (B) PSSC involves segregation of sister chromatids from an univalent in meiosis I. (C) Achiasmate non-disjunction (ND) originates by random segregation of two separated homologous chromosomes. (D) ‘True’ ND, probably caused by abnormal segregation of lineal medium-sized bivalents with a single distal chiasma.

Meiotic non-disjunction in humans: few differences between male and female

As observed in the current work and in human female meiosis (reviewed by Pellestor et al., 2006; Jones, 2008; Fragouli et al., 2011), spermatogenesis and oogenesis show resemblances with regard to meiosis I non-disjunction events, such as the similar contribution of PSSC and achiasmate non-disjunction to the genesis of aneuploidy and the predominant involvement of chromosomes of smaller size.

Unexpectedly, the level of numerical abnormalities in MII spermatozoa is similar to that described in fresh donated oocytes II from young women (Sandalinas et al., 2002) and three times higher than that reported in spermatozoa from healthy donors (Templado et al., 2005, 2011). This decrease of numerical abnormalities during spermatogenesis would imply the existence of a postmeiotic checkpoint at the spermatid stage (de Rooij and de Boer, 2003; Guichaoua et al., 2005) or in spermatozoa (Rodrigo et al., 2004), arresting specifically those germ cells with aneuploidy and diploidy. This postmeiotic checkpoint would also be responsible for the lower paternal origin of trisomies in humans (reviewed by Hassold et al., 2007), instead of differences in the incidence of meiotic chromosome segregation errors between males and females. Confirmation of the existence of this male checkpoint and the determination of the spermatogenesis stage in which it occurs has clinical implications in reproductive techniques, especially in ICSI of epididymal and testicular spermatozoa.
In conclusion, aneuploidy and diplody in spermatocytes II could be caused mainly by the absence of chiasmata at MI and, to a lesser extent, by errors in segregation of bivalents with a low chiasma number. As in female meiosis, the two main non-disjunction mechanisms during the first meiotic division in human spermatogenesis are PSSC and achiasmate non-disjunction, principally affecting chromosome 21 and sex chromosomes. The levels of numerical chromosome abnormalities in spermatocytes II and oocytes II from young women are similar, whereas spermatogonia show lower levels. This decrease would indicate the existence of a checkpoint during human spermatogenesis, which monitors and arrests those spermatocytes II with numerical abnormalities.

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**Authors’ roles**

Both authors, L.U. and C.T., have participated in study design, execution, analysis, manuscript drafting and critical discussion.

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**Conflict of interest**

None declared.

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Lacefield S, Murray AW. The spindle checkpoint rescues the meiotic segregation of chromosomes whose crossovers are far from the centromere. *Nat Genet* 2007;39:1273–1277.


