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SHORT COMMUNICATION

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# Molecular cytogenetics analysis with whole chromosome paint probes of sperm nuclei from a (13;15) Robertsonian translocation carrier

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**Abstract** Meiotic segregation of a Robertsonian translocation (13;15) was assessed in sperm nuclei using dual-color fluorescent in situ hybridization (FISH) with whole-chromosome paint probes. Most spermatozoa in the (13;15) translocation carrier resulted from alternate segregation. Although an increased frequency of unbalanced gametes was observed, spontaneous pregnancy led to the birth of a boy with a normal karyotype.

**Keywords** Fluorescent in situ hybridization · Robertsonian translocation · Spermatozoa

## Introduction

Robertsonian translocations (ROBs) are the most common recurrent chromosomal rearrangement observed in the general population. Translocations (13;14) and (14;21) are the most frequent, and all possible alternative non homologous recombinations are considered to be a rare event, including the (13;15) translocation (Gardner and Sutherland 1996). ROB is also one of the major chromosomal aberrations encountered in infertile men after numerical sex chromosome anomalies (Tuerlings et al. 1998). This structural rearrangement has variable impacts on spermatogenesis, including germ cell depletion by reduction of spermatocyte I survival potential (Guichaoua et al. 1990).

To date, sperm chromosome complements have been obtained in only one carrier of a (13;15) ROB after in vitro sperm penetration of golden hamster eggs (Pellestor 1990). Equal numbers of normal and balanced gametes have been obtained at a significantly higher rate compared with the number of unbalanced complements. However, to our knowledge, no data have been reported regarding the results of molecular cytogenetics analysis on spermatozoa from a t(13;15) translocation carrier.

The aim of our study was to evaluate spermatozoa meiotic segregation pattern from a carrier of a (13;15) translocation using dual-color fluorescent in situ hybridization (FISH) with whole-chromosome paint probes (WCP) in order to address the problem of specific genetic counseling.

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## Materials and methods

The (13;15) translocation carrier was a 38-year-old infertile male who presented with an oligoasthenozoospermia (sperm count: $10\times10^6$/ml, motility ($a$ + $b$): 25%). However, his wife began a spontaneous pregnancy of a foetus with a normal 46,XY karyotype. Six fertile healthy men aged between 25 and 41 years with normal semen parameters and a normal chromosome constitution were included in the study as a control group. All subjects gave their informed consent to participate in this study.

Meiotic segregation analysis was assessed on ejaculated spermatozoa using a dual-color FISH with WCP labeled with biotin for chromosome 13 and with fluorescein isothiocyanate (FITC) for chromosome 15 (STAR*FISH, Cambio, Adgenix, France). Hybridization was performed as described by Rives et al. (1998). The slides were examined at ×1,000 magnification using an epifluorescence microscope (DMRB; LEICA, Germany) with a digital imaging system (Mac Probe Version 3.3; Perceptive Scientific International Ltd., Chester, UK).

Spermatozoa with two fluorescent spots of different colors separated by at least the diameter of one signal were considered as normal with one chromosome 13 and one chromosome 15 and resulting from the alternate

meiotic segregation. Although two signals of different colors, close to one another in the nucleus, are more likely to represent the derivative in contrast separate signals are likely to represent two different chromosomes, we prefer to consider that sperm nuclei with two signals of different colors coupled one with the other are normal, with one chromosome 13 and one chromosome 15, or balanced with the derivative der(13;15). Moreover, taking into account the small size of the sperm nucleus, two separate chromosomes could be close to each other and appear as too close or even colocalizing signals. Sperm nuclei with two signals of the same color separated by more than the diameter of one signal were considered to contain two copies of the corresponding chromosome and to be disomic for this chromosome after meiotic nondisjunction or adjacent segregation. A spermatozoon was considered to be diploid or resulting from the 3:0 segregation if four fluorescent domains of different colors (two red and two green spots) were present. Some sperm nuclei were not included in the scoring: the signal of hybridization was sometimes completely absent, probably due to a failure of the hybridization; the fluorescent domains of some spermatozoa were occasionally very diffuse with a dispersed chromatin nuclei because the decondensation of the chromatin was too excessive; the overlapping nuclei were also excluded because the fluorescent signals could not be clearly distinguished. Data were statistically analyzed with Statview software (Abacus Concepts Inc., Berkeley, CA, USA) using a Chi-square test. A $p$ value $< 0.05$ was considered significant.

## Results

The meiotic segregation patterns of the ROB was determined in a total of 1,281 spermatozoa (Table 1, Fig. 1). In the control group, a total of 33,858 spermatozoa were assessed.

**Table 1** Fluorescent in situ hybridization (FISH) in dual color using whole-chromosome paint probes for chromosomes 13 and 15 in spermatozoa from the 45,XY,der(13;15) patient and the control group

| Combinations | Meiotic segregation | Frequency (%) | Total (%) | Control group (mean (%) ± SEM) |
|---|---|---|---|---|
| 13q/15q | Alternate (normal) | 51.36[a] | 78.13[b] | 98.46 ± 0.16[c] |
| 13q/15q or der(13q;15q) | Alternate (normal or balanced) | 26.77 | | |
| 13q/der(13q;15q) or 13q/13q/15q | Adjacent or meiotic nondisjunction | 4.68[d] | | 0.22 ± 0.02[e] |
| Including 13q/13q/15q | Disomy 13 (meiotic nondisjunction) | 1.56 | | |
| 15q/der(13q;15q) or 13q/15q/15q | Adjacent or meiotic nondisjunction | 2.72[f] | | 0.28 ± 0.06[g] |
| Including 13q/15q/15q | Disomy 15 (meiotic nondisjunction) | 0.62 | | |
| 13q | Adjacent or meiotic nondisjunction | 8.66[h] | | 0.16 ± 0.01[i] |
| 15q | | 4.13[j] | | 0.18 ± 0.03[k] |
| 13q/15q/13q/15q or der(13q;15q)/ der(13q;15q) or 13q/15q/der(13q;15q) | 3:0 or diploid | 1.63[l] | | 0.14 ± 0.01[m] |

[ac, bc, de, fg, hi, jk] $p < 0.0001$

[lm] $p < 0.006$

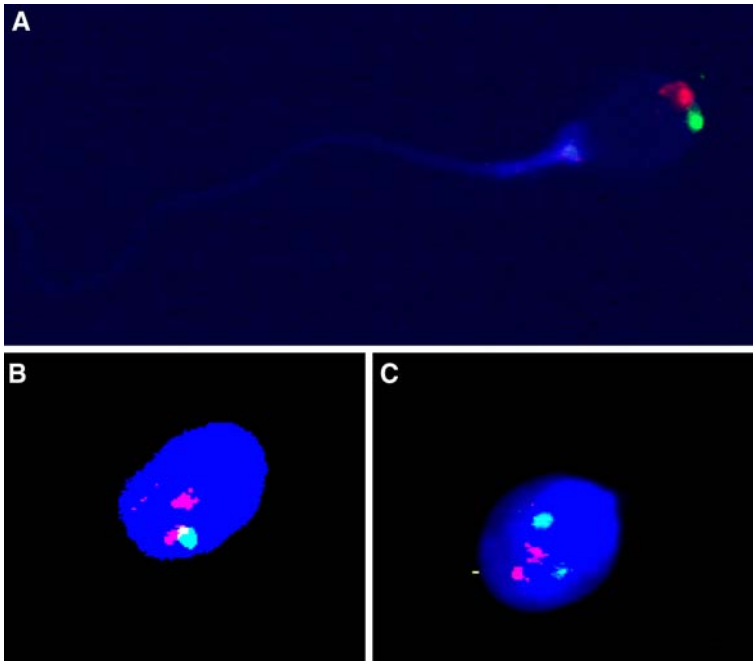


**Fig. 1** Spermatozoa from a t(13;15) carrier hybridized in dual-color fluorescent in situ hybridization (FISH) with whole-chromosome paint probes (WCP) for chromosome 13 (*red*) and for chromosome 15 (*green*). **a** A balanced der(13;14) or normal spermatozoon (alternate segregation). **b** A 13q disomic spermatozoon (adjacent segregation or meiotic nondisjunction). **c** A diploid or 3:0 segregation spermatozoon

A moderate decondensation of sperm nuclei was sufficient to obtain high hybridization efficiency without overswelling the sperm nuclei and impairing the morphology of the spermatozoa because long and short arms of chromosomes are less condensed than centromeres. Only sperm nuclei with bright and nonextended spots were scored. The number and relative size of specific chromosome domains can be accurately assessed in the majority of nuclei; all domains are not always clearly separable in these two-dimensional procedures. Two hybridization domains of the same color not clearly separated were excluded from the count.

The FISH analysis revealed that the rate of unbalanced segregation was 18.01% in the translocation carrier. The percentage of sperm nuclei with a normal or balanced karyotype was significantly higher than the percentage of unbalanced ones in the translocation carrier (78.13% versus 20.19%). However, the frequency of spermatozoa with a normal chromosome constitution (accurately identified and containing two fluorescent spots of different colors separated by at least the diameter of one signal) was significantly lower in the carrier (51.36%) compared with the control population (98.46%, $p < 0.0001$). The same result was observed when the global rate of sperm nuclei with a normal or balanced phenotype resulting from the alternate segregation was considered (78.13% versus 98.46%, $p < 0.0001$). The frequency of normal sperm nuclei (surely identified, 51.36%) was significantly higher compared with the frequency of balanced or normal sperm nuclei (not surely identified, 26.77%: $p < 0.001$) in the carrier.

Furthermore, the frequencies of disomic (7.40% versus 0.50%) or nullisomic (12.79% versus 0.34%) spermatozoa resulting from meiotic nondisjunction or adjacent segregation were significantly increased in the carrier ($p < 0.0001$). For chromosome 13, the nullisomy rate (8.66%) was significantly higher compared with the disomy rate in the carrier (4.68%, $p < 0.0001$) but not in the control group. For chromosome 15, no significant difference was observed between the two rates, as observed in control males. The disomy frequency for chromosomes 13 and 15 was estimated at 0.22% and 0.28% in the controls, significantly lower compared with the carrier ($p < 0.0001$). We were not able to differentiate diploid spermatozoa from spermatozoa resulting from the 3:0 segregation. Therefore, the frequency of diploidy was significantly increased compared with control males ($p = 0.006$).

## Discussion

During the pachytene stage of the first meiotic division, chromosomes involved in an ROB and their normal homologues form a trivalent. Alternate segregation produces balanced and normal gametes, respectively; adjacent and 3:0 segregation results in chromosomally unbalanced gametes. The meiotic segregation pattern of ROBs has been directly explored via karyotypes obtained after human sperm/hamster oocyte fusion assay or by sperm injection into mouse oocytes (Table 2). The (13;14) translocation is the most prevalent; however, other ROBs [t(14;21), t(15;22), t(21;22)] have also been reported, including one observation of a (13;15) translocation. As observed in our study, alternate segregation has been the most frequently described, ranging from 74% in a t(13;14) (Martin 1988) to 96% in a t(21;22) (Syme and Martin 1992) after human–hamster system and 86.7% in a t(13;14) (Ogawa et al. 2000) by sperm injection into mouse oocytes. Unbalanced gametes have been observed with various frequencies, reaching the maximum value of 26% in one study [t(13;14); Martin 1988).

FISH has also proven to be an alternative method for the evaluation of aneuploidy in spermatozoa, and this approach appears to be of particular interest for

**Table 2** Meiotic segregation of chromosomes in men carrying Robertsonian translocations (ROBs) implicated different nonhomologous (A and B) acrocentric chromosomes [t(A;B)] evaluated by sperm karyotypes

| Studies | Number of spermatozoa | Alternate (%) | | | Adjacent (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | Normal | Balanced | Nullisomy A−A | Nullisomy B−B | Disomy A−B + t(A;B) | Disomy B−A + t(A;B) | Diploid or 3:0 |
| Balkan and Martin (1983) t(14;21) | 24 | 70.8 | 16.7 | | | 13 | | |
| Pellestor et al. (1987) t(13;14) | 78 | 50 | 42.3 | | | 7 | | |
| Martin (1988) t(13;14) | 116 | 35.9 | 37.6 | | | 26 | | |
| Pellestor (1990) t(13;15) | 67 | 46.3 | 43.3 | | | 10 | | |
| Martin and Hildebrand (1992) t(15;22) | 115 | 42.6 | 47 | | | 10 | | |
| Syme and Martin (1992) t(21;22) | 149 | 74 | 70 | | | 3 | | |
| Ogawa et al. (2000) t(13;14) | 45 | 35.6 | 51.1 | | | 8.9 | | |

analyzing meiotic segregation pattern in sperm nuclei from reciprocal or ROB carriers. Most FISH studies concerning ROBs carriers have used locus-specific probes, with the exception of the study from Morel et al. (2001) performed with whole chromosome paint probes (WCP), as in the present study. We have previously demonstrated the specificity, sensitivity, and high hybridization efficiency of FISH with WCP for spermatozoa aneuploidy on sperm nuclei (Rives et al. 1998). The presence of interprotamine disulfite bridges is responsible for a particular structural organization of spermatozoon DNA and reduces the target of DNA hybridization. The condensation of spermatozoon DNA presented one potential advantage (the limitation of the hybridization domain to a discrete territory in sperm nucleus) and allowed us to develop FISH with WCP probes (Rives et al. 1998, 1999). The signal of DNA hybridization is more limited than in interphasic somatic cells, even if a preliminary decondensation with DTT at a high pH is performed. Another limitation with FISH using WCP is overlapping signals from the same color. Overlapping domains of hybridization can also be observed with FISH performed with alpha satellite centromeric probes. This event may underestimate the frequency of sperm nuclei carrying two 13q and/or 15q arms. An internal standardization was performed in our laboratory using WCP probes for all chromosomes in a population of fertile men with normal karyotypes and for chromosomes X, Y, 1, 13, 14, 18, 21, and 22 in a population of 50 infertile men with normal karyotypes (Rives et al. 1998, 1999). One major observation in the different studies performed in our laboratory using FISH with WCP probes is the absence of variability in sperm disomy in fertile men with normal karyotypes compared with the frequent heterogeneity of disomy reported with centromeric probes in the majority of FISH studies. In cases of ROBs carriers, this approach has allowed us to partially distinguish between normal and balanced spermatozoa (Morel et al. 2001). However, differentiation between spermatozoa resulting from 3:0 segregation and diploid nuclei was not possible with this method. Only three color FISH using another autosomal probe could show this distinction. However, with WCP, the overlapping fluorescent signals were increased. Furthermore, combination of WCP for chromosomes 13 and 15 with a centromeric probe for another autosome was not possible because decondensation used for WCP probes was not sufficient to obtain good hybridization efficiency of the centromeric probe.

Meiotic segregation of ROBs theoretically produces a higher frequency of chromosomally unbalanced gametes compared with the number of normal and balanced gametes. However, our results, as well as the data from different FISH studies, demonstrate the prevalence of normal or balanced sperm nuclei resulting from alternate segregation (for review, Morel et al. 2004). These data are also in agreement with those obtained after human sperm–hamster oocyte fertilization (Table 2): indeed, Pellestor (1990) reported a majority of balanced chromosome complements (87.4%) resulting from an alternate mode of segregation in spermatozoa of a (13;15) translocation carrier. These observations corroborate with the prevalent formation of a *cis* configuration in the meiotic trivalent structure of ROBs (Vidal et al. 1982; Luciani et al. 1984; Templado et al. 1984; Rosenmann et al. 1985), the *cis* configuration leading to the predominant alternate segregation. These authors also confirm that meiotic segregation of ROBs does not occur randomly. Alternate segregation mode has also been the most common mode of segregation observed after preimplantation genetic diagnosis (PGD) in embryos from ROBs carriers (Iwarsson et al. 2000; Alves et al. 2002).

A strong predominance of normal sperm nuclei compared with normal or balanced sperm cells was also observed in our (13;15) translocation carrier (51.36% versus 26.77%, respectively). We observed a rate of unbalanced spermatozoa to 18.01%. The published frequencies vary to a greater extent from one translocation to another and, for the same translocation, from one patient to another specifically for the frequent (13;14) translocation (for review, Morel et al. 2004). The variable distribution between balanced, normal, and unbalanced sperm nuclei may depend on the methodology used, the mode of ascertainment of the translocation, and the chromosomes involved in the translocation, as observed in reciprocal translocations. Furthermore, the observations from frequent ROBs could not perhaps be extrapolated to rare ROBs. Our patient presented a male infertility and sperm alterations whereas the patient from Pellestor (1990) had an history of unbalanced child with trisomy 13. This discrepancy remains unclear and justifies the evaluation of each ROB male carrier for its own imbalance rate in spermatozoa.

The adjacent mode of segregation theoretically generates, in ROB, an equal number of disomic and nullisomic gametes. This hypothesis was verified for chromosome 15 but not for chromosome 13 in our patient (Table 1). This excess of nullisomic 13 spermatozoa could be the consequence of low hybridization efficiency or to a lack of hybridization. We also suggest the occurrence of unequal hybridization efficiencies of both probes (Morel et al. 2001). However, in controls, the rate of nullisomic spermatozoa was lower compared with the rate of disomic spermatozoa with the same set of probes. An other explanation could be the possibility of spermatogenesis maturation arrest at meiosis II as regards preferentially disomic 13 cells leading to a relative decrease of disomic spermatozoa compared with nullisomic ones (Honda et al. 2000).

An excess of diploid spermatozoa was observed in our ROB carrier (1.63% versus 0.14% in the control group). However, we were not able to differentiate spermatozoa resulting from segregation 3:0 and diploid spermatozoa. The increased production of diploid spermatozoa observed in our ROB carrier is certainly the consequence of the abnormal chromosome constitution. Heterosynapses between unpaired regions of

some chromosomes are frequently observed in translocations carriers and may participate in the production of diploid spermatozoa (for review, Egozcue et al. 2000).

The FISH analysis should be proposed to each ROB carrier to personalize genetic counseling. Although the rate of imbalance was high, a spontaneous pregnancy occurred, resulting in a boy with a normal 46,XY karyotype.

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