Sperm aneuploidy and implications for genetic counseling in a pedigree of three t(1;3) balanced translocation carriers

L.-L. Li, Y. Dong, R.-X. Wang, N. An, X. Yun and R.-Z. Liu

Center for Reproductive Medicine, Center for Prenatal Diagnosis,
The First Hospital, Jilin University, Changchun, China

Corresponding author: R.-Z. Liu
E-mail: lrz410@126.com

ABSTRACT. A reciprocal translocation between the short arm of chromosome 1 and the long arm of chromosome 3 was observed in a pedigree of three carriers (proband, and his brother and mother). In this study, the three carriers had different clinical manifestations: the proband with infertility, his brother with spousal miscarriages, and his mother with no adverse reproductive history. Cytogenetic analysis of metaphase chromosomes was performed, and triple-color fluorescence *in situ* hybridization was applied to the detection of aneuploidy sperm related to the interchromosomal effect (ICE). An increase of aneuploidy of chromosome 21 in the proband and aneuploidy of chromosomes 13, 21, and Y in the brother were observed. Since patients with reciprocal translocations and spermatogenetic impairment are candidates, with their partners, for intracytoplasmic sperm injection, the study of the level of sperm aneuploidy rates would provide useful information for couples at risk, as well as contributing to a better understanding of the ICE.

Key words: Reciprocal translocation; Fluorescence *in situ* hybridization; Sperm aneuploidy rates; Infertility

INTRODUCTION

Balanced chromosomal translocations are often found as a cause of infertility (Martin, 2008). They are particularly frequent among couples experiencing recurrent miscarriages and among men with altered semen quality (Dohle et al., 2002). Reciprocal chromosomal translocations (RCTs) are produced by breakage and exchange of distal segments between non-homologous chromosomes. The incidence in the population is 1 in 712 newborn children (Nielsen and Wohlert, 1991). Unbalanced spermatozoa were detected in male carriers of balanced RCTs with a frequency of 18.6-80.7% (Benet et al., 2005). Whether or not meiotic association of the translocated chromosomes could interfere with the meiotic behavior of chromosomes not involved in the rearrangement, a phenomenon known as the interchromosomal effect (ICE), is still under debate. The issue of ICE is still very controversial because this effect has been found by some authors (Vegetti et al., 2000; Machev et al., 2005), but not by others (Estop et al., 2000; Oliver-Bonet et al., 2004). Furthermore, fertilization with an aneuploid sperm results in monosomy or trisomy in the fetus (Causio et al., 2002), and fetal aneuploidies are a major cause of pregnancy loss and fetal malformations (Li et al., 2002).

Here, we report a pedigree of three members with the RCT t(1;3)(p22;q29), and report on a sperm aneuploidy screening of chromosomes 13, 18, 21, X, and Y in the two males, one of whom has infertility problems and the other with recurrent miscarriages in his wife.

MATERIAL AND METHODS

Patients

The pedigree of the family is shown in Figure 1. The proband is a 25-year-old male, who presented with primary infertility having had two years of regular unprotected intercourse. The patient's medical history was unremarkable for infertility risk factors. Physical examination revealed normal penis and pubes. The left and right testicular volumes were both 18 mL. Three routine semen analyses, performed according to the World Health Organization guidelines (Meng et al., 2000), revealed severe oligoasthenoteratozoospermia. Reproductive hormone levels were normal for prolactin (193.30 µIU/mL; normal range: 86-324 µIU/mL), luteinizing hormone (5.6 mIU/mL; normal range: 1.7-8.6 mIU/mL), follicle-stimulating hormone (8.61 mIU/mL; normal range: 1.5-12.4 mIU/mL), testosterone (4.25 ng/mL; normal range: 2.8-8.0 ng/mL), and estradiol (19.75 pg/mL; normal range: 7.63-42.6 pg/mL). His mother had no adverse reproductive history, and his brother's wife had a history of three miscarriages. The seminal parameters for the proband (carrier 1) and his brother (carrier 2) are shown in Table 1. Appropriate voluntary written consent was obtained from the patient and his family. This study was approved by the Chinese Association of Humanitarianism and Ethics.

Chromosomal analysis

Cytogenetic investigations were performed on the patient's chromosomes obtained from peripheral blood lymphocytes, which were cultured in RPMI 1640 medium (GIBCO, Invitrogen, Carlsbad, CA, USA), phytohemagglutinin (Shanghai Yihua Medical Technology Co., Ltd., Shanghai, China), and fetal bovine serum (Beijing Dingguo Biotechnology, Beijing,

China) for 72 h, followed by treatment with 50 µg/mL colcemid. Metaphase chromosome spreads were studied by standard Giemsa (GTG) and replication (RBG) banding procedures.
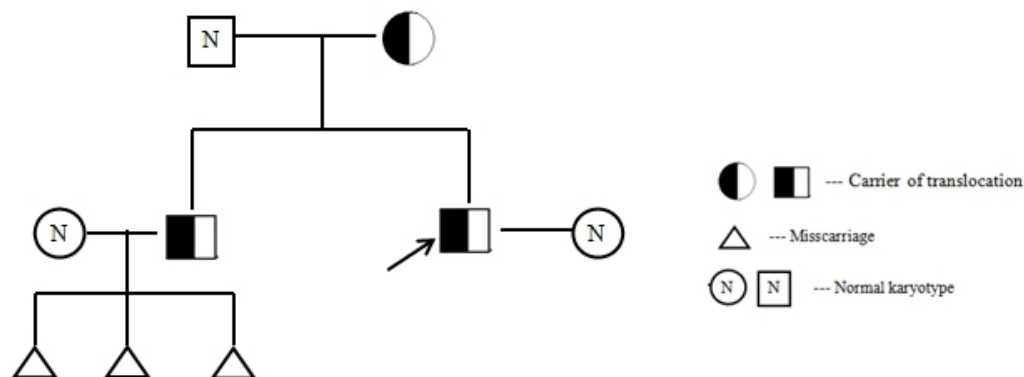
Figure 1. Pedigree of the family with the t(1;3) balanced translocation. The proband's mother is a carrier of a balanced chromosomal translocation. Her two sons both carry balanced reciprocal translocations. The proband (indicated with an arrow) presents primary infertility; his brother's wife has a history of three miscarriages.

Table 1. Parameters of sperm samples from the two t(1;3)(p22;q29) carriers.

Parameter	Value	
	Carrier 1 (Proband)	Carrier 2 (Brother)
Sperm volume (mL)	3.0	2.2
Sperm concentration (x 10^6/mL)	1.93	46.57
Progressive motility [A + B category (%)]	0.0	46.88
Good morphology (%)	2.0	15.0

Fluorescent *in situ* hybridization (FISH) analysis of spermatozoa

To evaluate aneuploidy frequency, FISH was performed according to Baccetti et al. (2003) on the sperm nuclei of the two male patients. A combination of centromeric probes for chromosomes 18, X, and Y (CSP18-Spectrum blue, CSPX-Spectrum green, and CSPY-Spectrum red, respectively; Beijing GP Medical Technologies, Beijing, China), and chromosome 13- and 21-specific probes (GLP13-Spectrum green and GLP21-Spectrum red, respectively; Beijing GP Medical Technologies) was used. At least 1000 nuclei per chromosome probe were scored. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows 17.0, SPSS Inc., Chicago, IL, USA). Semen samples from five healthy men with normal karyotypes and proven *in vivo* fertility were collected as controls. Chi-square analysis with Yates correction was performed to compare the aneuploidy frequencies of both male carriers, and to assess the differences with the fertile controls. A P value <0.05 was considered to be statistically significant.

Molecular deletion analysis

Multiplex polymerase chain reaction amplification of nine sequence-tagged site (STS)

markers was used to detect AZF region microdeletions on the Y chromosome, as previously described (Wang et al., 2010). These markers were: sY84 and sY86 for AZFa; sY127, sY134, and sY143 for AZFb; sY152, sY157, sY254, and sY255 for AZFc.

RESULTS

The G-banded karyogram of the proband, and his mother and brother revealed a balanced translocation between chromosomes 1 and 3. The exact positions of the breakpoints were at 1p22 and 3q29 (Figure 2a). An R-banded karyogram was also generated (Figure 2b). FISH sperm analysis was performed on the sperm nuclei from the proband and his brother. In total, 9307 spermatozoa were scored, and the disomy and diploidy frequencies of chromosomes 13, 18, 21, X, and Y were evaluated (Table 2). The sperm cells of the proband showed increased disomy frequencies for chromosome 21, with normal frequencies for the other chromosomes analyzed. However, the proband's brother showed a significantly elevated level of disomy 21 (1.38% of all cell nuclei analyzed), as well as elevated disomy of chromosomes 13 (0.86%) and Y (0.18%), compared to the control groups. The two patients' overall diploidy rate was not markedly increased. At the molecular level, no microdeletions were detected in the AZF region on the proband's Y chromosome using the nine STS markers.
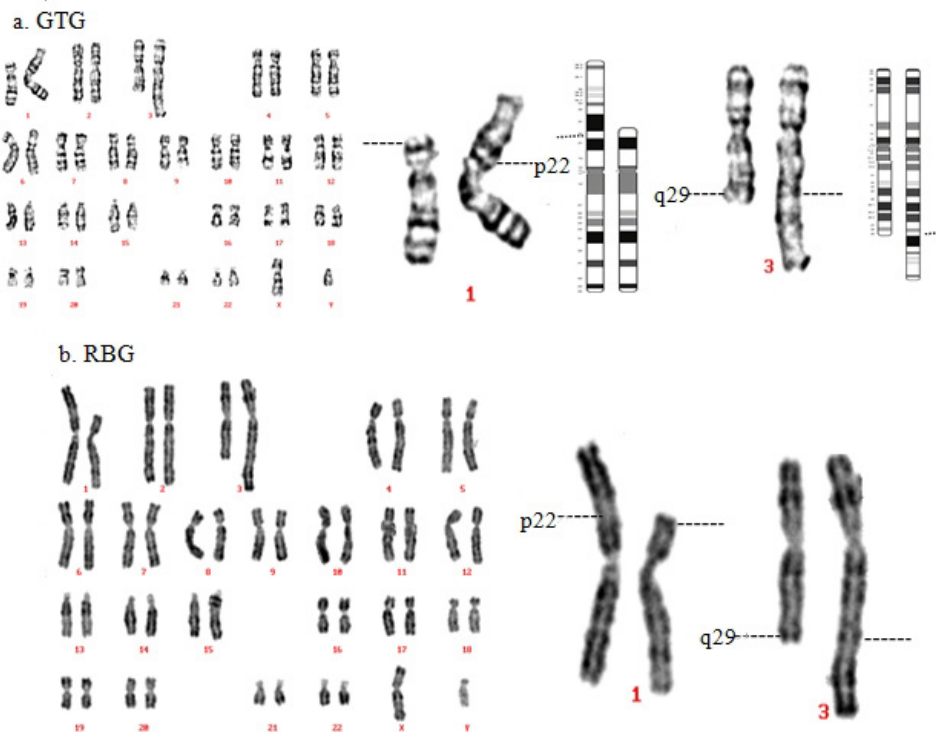
Figure 2. Proband karyograms. **a.** G-banded karyotype (GTG) of the proband with a balanced translocation 46,XY,t(1;3)(p22;q27), and chromosome ideograms. **b.** R-banded karyotype (RBG) of the proband. Chromosome numbers are indicated in red.

Table 2. Incidence of sperm disomy and diploidy of chromosomes 13, 18, 21, X, and Y in two male carriers of reciprocal translocations.

Patients	Disomy frequency (%)						Diploidy (%)
	13,13	18,18	21,21	X,X	Y,Y	X,Y	2x (13,18)
Carrier 1	0.176	0.112	0.546*	0.071	0.083	0.209	0.299
Carrier 2	0.860*	0.152	1.38*	0.081	0.179*	0.178	0.306
Control group	0.154 ± 0.02	0.103 ± 0.02	0.245 ± 0.04	0.060 ± 0.01	0.048 ± 0.01	0.137 ± 0.04	0.277 ± 0.03

*Significantly higher than in the control group (P < 0.05, chi-square test). Mean frequency ± SD for the control group.

DISCUSSION

Balanced translocations can be transmitted through the generations, and a high incidence of unbalanced gametes connected with infertility and miscarriage was observed in the pedigree presented in this study. The interfamilial variation in meiotic segregation of the same translocation has been analyzed in previous studies, and in each of these families, similar segregation profiles have been detected (Estop et al., 1992; Rousseaux et al., 1995; Cora et al., 2002; Anton et al., 2004; Morel et al., 2004; Wiland et al., 2007). Although the models of meiotic segregation for RCTs require equal proportions of complementary segregants, which result from all types of recombination events, this phenomenon of equal segregant proportions has not been documented. To date, there is no clear explanation why it has not seen equal segregant proportions; some authors have discussed the possibility of differences between the frequencies of complementary products, which could result from the viability of the spermatocytes and spermatids according to their chromosomal contents, and could represent evidence for the preferential selection of certain segregants (Blanco et al., 1998). Although in our study, the male t(1;3)(p22;q29) carriers have not been examined at the level of the meiotic segregation patterns of spermatozoa, we can infer that the two male carriers had similar meiotic segregation patterns as well as similar translocations. It is generally believed that the model of pairing of balanced chromosomal rearrangements seems to be directly related to the prognosis for carrier fertility and possible anomalies in his progeny. However, the observations that spermatogenesis and/or reproductive failures affect only some of the male carriers with the same familial translocation, as described in our case, as well as the differences in the sperm parameters between the proband and his brother, and the differences in the reproductive outcomes between the two male carriers and their mother cannot be explained on the basis of meiotic segregation patterns alone. The differences in reproductive failure described above may be the result of additional genetic or environmental factors.

It is known that approximately 30% of the carriers of different reciprocal translocations exhibit aneuploidy of some chromosomes other than those engaged in the structural rearrangement (Gianaroli et al., 2002). Due to their high frequency of trisomy in live offspring, we chose chromosomes X, Y, 13, 18, and 21 to be investigated for whether the t(1;3)(p22;q29) translocation had positive ICEs. In our results, the proband showed an increase in aneuploidy of chromosome 13, and his brother showed increased aneuploidy not only of chromosome 13 but also of chromosomes 21 and Y. Although both patients showed increased aneuploidy for chromosome 13, the different aneuploidy rates for chromosomes 21 and Y made it difficult to argue for a clear ICE effect in relation to this translocation. It is known that men with a normal

somatic karyotype but abnormal sperm parameters and/or reproductive failure (also with miscarriages) have significantly higher rates of aneuploidy than fertile controls (Shi and Martin, 2001). Therefore, in the present study, we could not exclude the possibility that the higher frequency of chromosome 13 disomy in the proband originated from his poor semen quality.

During the last decade, the chances of translocation carriers to have a healthy child have increased due to the introduction of *in vitro* fertilization and embryo transfer, along with preimplantation genetic diagnosis (PGD) of the translocations, which enables the selection of only embryos with normal/balanced karyotypes for transfer (Munné, 2005). Other studies, however, have found that in couples including a carrier of a structural chromosome rearrangement, approximately one-third of the miscarriages were found to have an unbalanced structural chromosome rearrangement, and two-thirds were euploid, polyploidy, or aneuploid, accounting for the majority of the miscarriages (Stephenson and Sierra, 2006). Therefore, the use of PGD should be indicated in cases where the miscarriage rate is expected to be high in a natural pregnancy, for others who have no experience of miscarriage we recommend through natural pregnancy. In this study, the proband had severe oligoasthenoteratozoospermia, and we suggested that he consider PGD to reduce the risk of miscarriage; and that his brother and his wife try natural pregnancy again and undergo prenatal diagnosis if pregnant, since the brothers' mother was also a carrier of the same t(1;3)(p22;q27) without a history of miscarriage.

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