

ORIGINAL ARTICLE

ANALYSIS OF MEIOTIC SEGREGATION PATTERNS AND INTERCHROMOSOMAL EFFECTS IN SPERM FROM 13 ROBERTSONIAN TRANSLOCATIONSWang B^{1,*}, Nie B^{1,*}, Tang D^{2,*}, Li R^{3,*}, Liu X¹, Song J¹, Wang W¹, Liu Z^{1,**}

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****Corresponding Author:** Dr. Zhi Liu, Department of Clinical Laboratory, Hubei Maternal and Child Health Hospital, 745 WuLuo Road, Wuhan, Hubei, People's Republic of China. Tel: +86-27-8716-9190. Fax: +86-27-8716-9097. E-mail: wangbo1005@163.com.**ABSTRACT**

The frequency of the Robertsonian (ROB) translocation in newborn babies is approximately one in 1000. Robertsonian translocation is an unusual type of chromosome rearrangement caused by two particular chromosomes joining together. The aim of the study was to analyze the segregation of the ROB translocations in 13 male carriers, and to verify a possible inter-chromosomal effect (ICE) of the ROB translocation on chromosomes 18, X, and Y. Thirteen male patients were included in the study. Multicolor fluorescent *in situ* hybridization (FISH) was used to analyze chromosomes 13, 14, 15, 21, 22, 18, X and Y in sperm. Among the heterozygous ROB translocation carriers, the frequency of normal/balanced spermatozoa resulting from alternate segregation varied between 70.4 and 85.2%. The frequency of unbalanced spermatozoa resulting from adjacent segregation varied between 14.8 and 29.6%. Increased frequencies of aneuploidy for a sex chromosome were found in 10 ROB translocation carriers (P2-P8, P10-P12). Increased frequencies of aneuploidy for chromosome 18 were found in 10 ROB translocation carriers (P3-P9, P11-P13). In addition, increased frequencies of diploid were found in 11 ROB translocation carriers (P2-P9, P11-P13). Among the homozygous ROB translocation carriers, the rate of balanced spermatozoa was 99.7% and the frequency of unbalanced spermatozoa was 0.3%. However, the frequencies of aneuploidy for a sex chromosome and chromosome 18 were normal. Despite the high number of normal/balanced

frequencies, there remained many unbalanced spermatozoa resulting from alternate segregation. The ROB translocation carriers may be at an increased risk for ICE. Robertsonian translocation homozygosity could be seen as a potential speciation in humans with 44 chromosomes.

Keywords: Evolution; Interchromosomal effects; Meiotic segregation; Robertsonian (ROB) translocation homozygosity; Sperm fluorescence *in situ* hybridization (FISH).

INTRODUCTION

The frequency of the Robertsonian (ROB) translocation in newborn babies is approximately one in 1000 [1]. The ROB translocation is an unusual type of chromosome rearrangement caused by two particular chromosomes joining together. In humans, it occurs in the five acrocentric chromosomes, such as chromosomes 13, 14 and 15 (group D), and 21 and 22 (group G). During a ROB translocation, the participating chromosomes break at their centromeres and the long arms fuse to form a single chromosome with a single centromere. The short arms also join to form a reciprocal products that typically contain nonessential genes and are usually lost within a few cell divisions. The most common ROB translocation in humans is between chromosomes 13 and 14, constituting 75.0% of all ROB translocations [1]. Since ROB translocation carriers have a balanced chromosomal complement, they may never be aware of their unusual chromosome rearrangement. Therefore, ROB translocation can be passed down in the family for many generations without detection.

In ROB translocations, at the end of meiosis I, segregation of the translocated and non translocated chromosomes from the two different implicated chromosome pairs, lead to the formation of either balanced (alternate segregation mode) or unbalanced (adjacent 1, adjacent 2, and 3:0 segregation modes) gametes that can segre-

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gate in different ways at anaphase [2,3]. Only alternate segregation produces normal/balanced karyotypes. The other segregation modes (adjacent 1, adjacent 2, 3:0) produce unbalanced gametes with disomies and nullisomies of chromosomes involved in ROB translocations. It is well-known that meiotic tetravalent configuration tends to segregate in an alternate way [4], resulting in the production of normal/balanced spermatozoa. However, a certain percentage of unbalanced gametes deriving from adjacent segregation are also produced, and could be responsible for the miscarried or the severely affected aneuploidy offspring frequently born to these carriers.

Analysis of the chromosomal constitution in sperm of ROB translocation carriers is of great interest to assess the risk of unbalanced offspring for genetic counseling. Sperm fluorescence *in situ* hybridization (FISH) using chromosome specific probes has proven to be a useful technique to determine the meiotic segregation, especially in translocation carriers [5-9]. During the last decade, meiotic segregation in spermatozoa has been repeatedly studied in male carriers of ROB translocations. Despite the large variations, the rate of unbalanced gametes is generally not conclusive enough for genetic counseling. Although most studies showed strong prevalence of alternate segregation, which lead to balanced spermatozoa, one recent study showed a high percentage of unbalanced spermatozoa in two ROB translocation carriers [10].

More recently, inter-chromosomal effects (ICE) have been reported in several chromosome pairs in ROB translocations. Inter-chromosomal effects refer to a disturbance of meiosis, where rearranged chromosomes disrupt disjunction and distribution of chromosome pairs not involved in the rearrangement. The concept of ICE was first postulated by Lejeune [11], who noticed an excess of carriers of balanced reciprocal translocations among the fathers of children with Down syndrome. Since then, contradictory data have been reported in ROB translocation carriers. Several studies have found such an ICE in male carriers of ROB translocations [12,13], but others did not [14,15]. The aims of this study were to analyze the segregation pattern of ROB translocation in 13 male carriers, and evaluate the occurrence of ICE on chromosomes 18, X and Y in these carriers.

MATERIALS AND METHODS

Patients. Thirteen male carriers of ROB translocations (designated P1-13) consulting for infertility or repeated abortions were included in this study. Twelve were heterozygous ROB translocation and one was a homozygous ROB translocation. Except P1 and P2 (P2 was P1's

father), all the patients were unrelated. Three males with normal 46,XY karyotype and normal sperm characteristics were recruited as controls. The local ethics committee approved this protocol and all patients and controls had previously given informed consent for the study.

Sperm Preparation, Fluorescent *in Situ* Hybridization and Scoring. All semen samples were first analyzed to evaluate volume, concentration and motility according to the World Health Organization (WHO) criteria [16]. After semen analysis, sperm with progressive motility was isolated and washed twice in phosphate buffered saline (pH 7.4) by centrifugation at 1500 rpm for 11 min. Final pellets were fixed with 5 mL of acetic acid/methanol mixture (1:3) for at least 30 min. at 4 °C. Aliquots (40-50 µL) of the resulting suspension of nuclei were smeared on cold pre-cleaned slides. Nuclei decondensation was performed in 1 N NaOH for 2 min. After dehydration in ethanol series (70.0, 90.0, 100.0%), denaturation was performed in 0.25% formamide in 2 × saline sodium citrate (SSC) followed by overnight hybridization with a combination of commercially available probes.

To assess the segregation behavior of each rearrangement, a dual-color FISH using locus-specific probes and/or telomere probes and/or chromosome enumeration probes, was carried out on spermatozoa of the translocation carriers. Below we list the probes that we used to identify the segregation products for each karyotype. For a der(13;14) carrier, LSI13 (13q14, Spectrum Green) and Tel14 (14q32.33, Spectrum Red) were used. For a der(14;15) carrier, Tel14 (14q32.33, Spectrum Red) and Tel15 (15q26.3, Spectrum Green) were used. For a der(14;21) carrier, Tel14 (14q32.33, Spectrum Red) and LSI21 (21q22.13-q22.2, Spectrum Green) were used. For a der(13;22) carrier, LSI13 (13q14, Spectrum Green) and LSI22 (22q12.3, Spectrum Red) were used. For a der(21;22) carrier, LSI21 (21q22.13-q22.2, Spectrum Green) and LSI22 (22q12.3, Spectrum Red) were used. Regarding ICE evaluation, triple-color FISH was performed using the probe mixture including commercial satellite (DNA) probes for chromosomes 18, X and Y (CEP 18, Spectrum Blue/CEP X, Spectrum Green/CEP Y, Spectrum Red) from Vysis (Abbott Park, IL, USA).

Post-hybridization washes included 2 min. in 0.4 × SSC/0.3% NP-40 (pH 7) at 72 °C, followed by 1 min. in 2 × SSC/0.1% NP-40 (pH 7) at room temperature. Slides were covered with DAPI II (Vysis). Only intact spermatozoa bearing a similar degree of decondensation and clear hybridization signals were scored. Disrupted or overlapping spermatozoa were excluded from analysis. Only slides with hybridization efficiency of 99.0% and more were analyzed. One thousand sperm nuclei per patient were analyzed.

Statistical analyses. The χ^2 test was used to compare the frequencies of segregation products. A *p* value of less than 0.05 was regarded as statistically significant.

RESULTS

The age of our patients ranged from 25 to 48 years with a mean of 38.6 years. The karyotype and sperm examination of patients and controls are given in Table 1. According to WHO criteria, all our patients, except P1,

had very poor semen parameters. The karyotyping analysis identified a previously undescribed balanced human karyotype 44,XY,der(14;15)(q10;q10),der(14;15)(q10;q10) (Figure 1). Interestingly, in P1, we saw a sperm (arrow) with four fluorescence signals (two red and two green). This means the sperm has two derivative chromosomes of der(14;15), but we could not tell if the sperm was a disomy of derivative chromosome or diploid (Figure 2). In future studies, we will employ triple-color FISH (for example, the probe mixture will consist of chromosomes 14, 15 and 18)

Table 1. Cytogenetic and spermiologic results of patients and controls.

Patient	Karyotype	Age (years)	Sperm Concentration (10 ⁶ /L)	Motility ^a (a+b)%
P1	44,XY,der(14;15)(q10;q10),der(14;15)(q10;q10)	25	56.0	53.0
P2	45,XY,der(14;15)(q10;q10)	48	13.0	22.0
P3	45,XY,der(13;14)(q10;q10)	29	19.0	25.0
P4	45,XY,der(13;14)(q10;q10)	31	22.0	17.0
P5	45,XY,der(13;14)(q10;q10)	27	38.0	21.0
P6	45,XY,der(13;14)(q10;q10)	35	57.0	59.0
P7	45,XY,der(13;14)(q10;q10)	33	89.0	38.0
P8	45,XY,der(13;14)(q10;q10)	30	35.0	29.0
P9	45,XY,der(14;21)(q10;q10)	36	40.0	35.0
P10	45,XY,der(14;21)(q10;q10)	32	37.0	36.0
P11	45,XY,der(14;21)(q10;q10)	26	42.0	45.0
P12	45,XY,der(13;22)(q10;q10)	26	28.0	39.0
P13	45,XY,der(21;22)(q10;q10)	29	31.0	47.0
C1	46,XY	35	77.0	44.0
C2	46,XY	27	62.0	47.0
C3	46,XY	33	43.0	39.0

^a Sperm motility was graded from a to d, according to the WHO manual criteria, as follows. Grade a (fast progressive) sperms are those that swim forward fast in a straight line, like guided missiles. Grade b (slow progressive) sperms swim forward, but either in a curved or crooked line, or slowly (slow linear or non linear motility). Grade c (non progressive) sperms move their tails, but do not move forward (local motility only). Grade d (immotile) sperms do not move at all. The (a+b)% represent the percentage of sperm that can move forward normally.

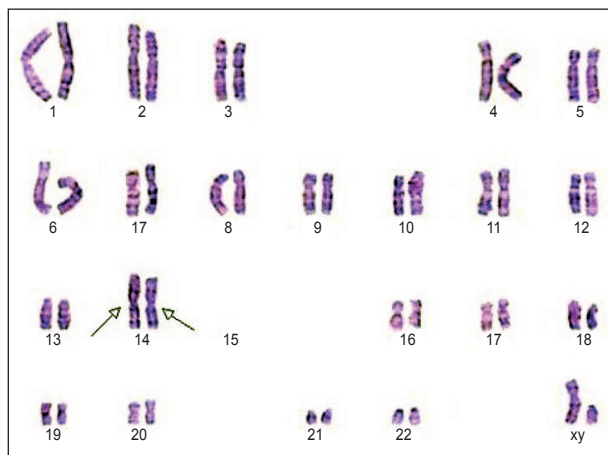


Figure 1. Karyotype of P1: 44,XY,der(14;15)(q10;q10),der(14;15)(q10;q10), carrying a disomy for the ROB translocation chromosome (highlighted by arrows).

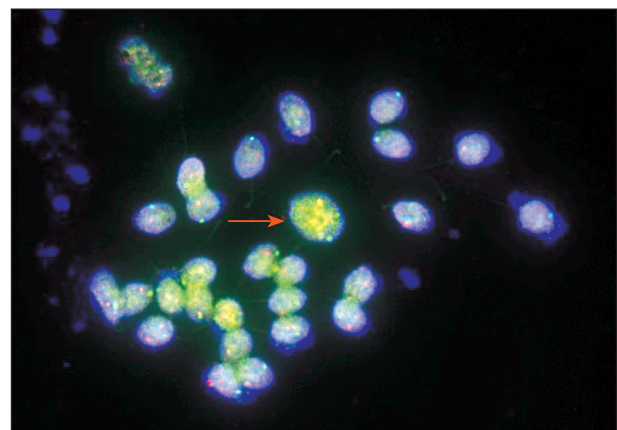


Figure 2. Sperm cells after hybridization with TelVysion 14q32.33 (Spectrum Red) and TelVysion 15q26.3 (Spectrum Green). The sperm highlighted with a red arrow may be a diploid sperm.

Table 2. The number of spermatozoa scored, the alternate mode of segregation, incidence of sperm nullisomy, disomy and 3:0/diploid for the chromosomes involved in the Robertsonian translocation in 13 Robertsonian translocation carriers and three controls.

	Normal/Balanced	Nullisomy A	Disomy A	Nullisomy B	Disomy B	3:0 or Diploid	Unbalanced	Total
P1	997	2	0	0	0	1	3	1000
P2	799	41	49	55	45	11	201	1000
P3	852	24	57	34	26	7	148	1000
P4	809	68	30	55	29	9	191	1000
P5	843	40	39	27	41	10	157	1000
P6	757	55	37	94	42	15	243	1000
P7	837	29	66	34	21	13	163	1000
P8	782	75	61	49	22	11	218	1000
D/D mean	811	47.0	48.0	50.0	32.0	11.0	189.0	
P9	775	41	50	70	45	19	225	1000
P10	721	66	37	110	45	21	279	1000
P11	704	47	56	73	95	25	296	1000
P12	719	69	75	62	58	17	281	1000
D/G mean	730	56.0	55.0	79.0	61.0	21.0	270.0	
P13	768	47	51	42	80	12	232	1000
C1	995	0	2	1	1	1	5	1000
C2	997	1	0	2	0	0	3	1000
C3	995	0	2	0	1	2	5	1000
Control mean	996	0.3	1.3	1.0	0.7	1.0	4.3	

A: first chromosome involved in the ROB translocation; B: second chromosome involved in the ROB translocation; D/D mean: the mean of P2-P8 (ROB translocation between D/D groups); D/G mean: the mean of P9-P12 (ROB translocation between D/G groups); Control mean: the mean of the three controls (C1-C3).

Table 3. Incidence of sperm nullisomy, disomy and diploid for chromosomes 18, X and Y in 13 Robertsonian translocation carriers and three controls.

	Normal/Balanced	Nullisomy 18	Disomy 18	Nullisomy Sex Chromosome	Disomy Sex Chromosome	3:0 or Diploid	Unbalanced	Total
P1	994	0	1	2	3	0	6	1000
P2	949	3	2	15	10	21	41	1000
P3	899	6	5	22	21	47	101	1000
P4	952	4	5	17	12	10	48	1000
P5	913	11	9	13	15	39	87	1000
P6	947	7	2	15	17	12	53	1000
P7	955	9	12	10	5	9	45	1000
P8	901	21	13	24	19	22	99	1000
D/D mean	931	8.7	6.9	16.6	14.1	22.9	67.7	
P9	976	3	4	4	2	11	24	1000
P10	985	2	1	3	6	3	15	1000
P11	963	6	4	9	11	7	37	1000
P12	944	7	5	8	11	25	56	1000
D/G mean	967	4.5	3.5	6.0	7.5	11.5	33.0	
P13	977	7	6	1	4	5	23	1000
C1	995	0	2	1	1	1	5	1000
C2	997	1	0	2	0	0	3	1000
C3	991	0	2	3	1	3	9	1000
Control mean	994	0.3	1.3	2.0	0.7	1.3	5.7	

for the detection of normal/balanced or unbalanced sperm to distinguish between disomy and diploid.

The results of the segregation analysis are given in Table 2 and Table 3. Among the 13 ROB translocation carriers, one was homozygous (P1). The remainder were heterozygous (P2-P13). The frequency of balanced spermatozoa was 99.7% in P1, while the frequency of unbalanced spermatozoa was 0.3%, which was similar to the controls [17-21] (Table 2).

In P2-P13, the frequency of normal/balanced spermatozoa resulting from alternate segregation varied between 70.4 and 85.2%. The frequency of unbalanced spermatozoa resulting from adjacent segregation varied between 14.8 and 29.6%. The frequency of unbalanced spermatozoa was significantly higher than P1 and the controls ($p < 0.05$).

To evaluate the occurrence of ICE, the nullisomy, disomy and diploid frequencies for chromosomes 18, X and Y are summarized in Table 3. Increased frequencies of aneuploidy for a sex chromosome were observed in 10 ROB translocation carriers (P2-P8, P10-P12), increased frequencies of aneuploidy for chromosome 18 were observed in 10 ROB translocation carriers (P3-P9, P11-P13). In addition, increased frequencies of diploid were found in 11 ROB translocation carriers (P2-P9, P11-P13). Increased frequencies of unbalanced sperms were found in heterozygous carriers (P2-P13). In contrast, no ICE was detected in P1.

DISCUSSION

Sperm chromatin is a highly organized, compact structure consisting of DNA and heterogeneous nucleoproteins, which are essential for the accurate transmission of genetic information to offspring [22]. Sperm FISH is most commonly used to determine the proportion of aneuploidy present in sex chromosomes and autosomes of infertile men. Furthermore, it can also quantify the probability of transmitting aneuploidies and complex chromosomal rearrangements, such as translocations and inversions [22]. As a result, sperm FISH analysis is increasingly included in infertility diagnostic protocols, providing useful information for genetic consulting. In our study, we performed sperm FISH analyses on 13 male ROB translocation carriers.

In our study, we had one homozygous carrier (P1) and 12 heterozygous carriers (P2-P13). In P1, the frequency of unbalanced spermatozoa was only 0.3% that was similar to controls and is consistent with previous publications [5-7]. In contrast, in P2-P13, the frequency of normal/balanced spermatozoa resulting from alternate segregation varied between 70.4 and 85.2% (Table 2). This is consistent

with previous studies showing that alternate segregation is predominant in sperm of Robertsonian translocation carriers [11,20,21]. The high prevalence of the alternate segregation is presumably due to *cis*-configuration of the trivalent during meiosis, which favors an alternate segregation in all ROB translocations [21-24]. However, the frequency of unbalanced spermatozoa resulting from adjacent segregation varied between 14.8 and 29.6% (Table 2), which is significantly higher than controls. Robertsonian translocations consist of a fusion of two acrocentric chromosomes at the centromere level to form a derivative chromosome (group D: 13, 14 and 15; group G: 21 and 22). Robertsonian translocation can be separated into three subtypes: D/D, D/G and G/G translocation. Patients P2-P8 were D/D ROB translocation carriers; P13 was a G/G ROB translocation carrier, and P9-P12 were D/G carriers. The frequency of unbalanced spermatozoa was highest in the D/G carriers and lowest in the D/D carriers and the differences between D/D and D/G, D/D and G/G were significant ($p < 0.05$). In contrast, the difference between G/G and D/G was not significant ($p > 0.05$). This is consistent with previous publications [6,17]. We speculated that in D/D ROB translocation, the derivative chromosome is a metacentric chromosome, so it is prone to alternate segregation rather than adjacent segregation at the end of meiosis I. In the G/G ROB translocation, the derivative chromosome is a metacentric chromosome, but the derivative chromosome is shorter than the derivative chromosome of D/D ROB translocation, so the rate of adjacent segregation is higher than D/D ROB translocation. In the D/G ROB translocation, the derivative chromosome is a submetacentric chromosome, it is more chaotic than D/D and G/G ROB translocations at the end of meiosis I, so the rate of unbalanced spermatozoa is significantly higher than D/D and G/G ROB translocations.

The homozygous ROB translocation carrier (P1) is healthy and has a balanced chromosomal complement. Assessment of a semen sample from P1 showed normal sperm number and morphology. Given his karyotype of 44,XY,der(14;15)(q10;q10),der(14;15)(q10;q10), we hypothesized that the person's sperm karyotype to be consistently 22,X,der(14;15) and 22,Y,der(14;15), then, our hypothesis was proven by this research. Most of the sperm from P1 are balanced haploid (not normal haploid) (Table 2, Table 3). Homozygous ROB translocations in man have been described before. A fetus with two t(14;21) chromosomes was found by Dallapiccola *et al.* [25]. The related parents were heterozygous for the same translocation. Martinez *et al.* [26] described three adult siblings homozygous for t(13;14). Their parents were first cousins and both

were heterozygous carriers. Rajangam *et al.* [27] found a unique DS karyotype 45,XY,der(14;21)pat,der(14;21)mat,+21mat. While translocation heterozygosity is associated with meiotic disturbances that cause infertility and subfertility, translocation homozygosity should not, at least in theory, have any effect on meiosis [28]. In our study, we have one patient with homozygous ROB translocation.

Inter-chromosomal effect remains controversial in the literature [29]. Some publications have indicated its relevance [12,13], while others showed no evidence of ICE in ROB translocation carriers [9,18]. Inter-chromosomal effect could be explained by the formation of heterosynapses between chromosomes involved in the translocation and the sex vesicle, which could also involve other chromosomes [11,29]. In our study, we observed higher frequency of aneuploidy for the sex chromosome in 10 ROB translocation carriers (P2-P8, P10-P12) and higher frequencies of aneuploidy for chromosome 18 in 10 ROB translocation carriers (P3-P9, P11-P13). In addition, increased rates of diploid were found in 11 ROB translocation carriers (P2-P9, P11-P13). Increased rates of unbalanced sperms were found in all ROB translocation heterozygote (P2-P13). The incidence of spermatozoa with nullisomy, disomy and diploid for the sex chromosomes of ROB translocation heterozygotes (P2-P13) was significantly higher compared to that of ROB translocation homozygosity (P1) ($p < 0.05$). Robertsonian translocation rearrangements are common chromosomal changes that can lead to rapid and efficient reproductive isolation between karyotypically similar populations, especially when many ROB metacentric chromosomes display monobrachial homologies [30]. In the case of *Muntjac* deer, also called Cervidae, which are of great interest to evolutionary biologists and cytogeneticists because of the considerable diversity of their karyotypes, despite their morphological similarity [28].

To conclude, our study confirmed that alternate segregation is dominant in ROB translocation carriers. However, despite the high number of normal/balanced spermatozoa, there are still a lot unbalanced spermatozoa resulting from adjacent mode of segregation. Our study provides further evidence of ICE on chromosome X, Y and 18 in ROB translocation carriers. Therefore, preimplantation genetic diagnosis (PGD) is recommended for ROB translocation carriers to minimize the risk of having an affected child or the distress of pregnancy termination, and to reduce the risk of miscarriage due to abnormal segregation of the translocation [31-34]. Since the proband is phenotypically normal with normal fertility, we considered the chromosomal rearrangement of the person to be a balanced polymorphism [35]. The aberration can provide

material for evolution. The establishment of a new human subspecies with a diploid complement of 44 chromosomes could occur if a small population with the karyotype of the proband undergoes long-term reproductive isolation [36].

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