Analysis of meiotic segregation patterns and interchromosomal effects in sperm from six males with Robertsonian translocations

Yongjian Chen · Jin Huang · Ping Liu · Jie Qiao

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Abstract

Purpose To provide more genetic information about meiotic segregation behavior and the possibility of interchromosomal effects (ICE) in spermatozoa from carriers of Robertsonian (Rob) translocations.

Materials and methods Meiotic segregation behavior in spermatozoa from six carriers of Rob translocations, four t(13;14), one t(14;22) and one t(13;21), was investigated by dual fluorescence in-situ hybridization (FISH). Aneuploidy for chromosomes 18, X and Y was studied by triple FISH.

Results The rate of normal/balanced spermatozoa resulting from alternate segregation ranged from 78.14 to 86.88%. The frequency of unbalanced spermatozoa resulting from adjacent segregation varied between 11.70 and 19.53%. The higher frequencies of aneuploidy for sex chromosome were observed in three Rob translocation carriers. In addition, the increased rates of diploid were found in two t(13;14) carriers.

Conclusions Alternate segregation is dominant in the different types of Rob translocations. Some carriers may be at an increased risk for ICE.

Keywords Fluorescence in-situ hybridization · Interchromosomal effects · Meiotic segregation · Robertsonian translocation · Sperm

Introduction

Robertsonian (Rob) translocations are one of the most common structural chromosomal rearrangements and occur in approximately 2–3% in infertile men. Rob translocations are characterized by centric fusion between two acrocentric chromosomes (pairs number 13, 14, 15, 21, and 22), resulting in the formation of a chromosome composed of the long arms of two acrocentric chromosomes and subsequent loss of their short arms. As a consequence of this fusion, a carrier of a Rob translocation has only 45 chromosomes. Translocation between chromosomes 13 and 14 is the most frequent one in humans, estimated to be approximately 75% of all Rob translocations. The t(14;22) and t(13;21) are two rare Rob translocations, comprising about only 1.2 and 2% of all detected Rob translocations, respectively [1].

In general, male carriers of Rob translocations are phenotypically normal, but do have more frequent fertility problems. The carriers produce increased numbers of unbalanced sperm resulting in repeated abortions, chromosomally unbalanced offspring or infertility.

Analysis of the chromosomal constitution in sperm of Rob translocation carriers is of great interest for assessing the risk of unbalanced offspring and adapting genetic counseling. During the last decade, meiotic segregation in spermatozoa has been repeatedly studied in male carriers of Rob translocations [2–12]. Most of these studies showed strong prevalence of alternate segregation in gametes.

An increased aneuploidy for sex chromosome observed in three Robertsonian translocation carriers suggests that an interchromosomal effect is likely in some carriers.

Y. Chen (✉) · J. Huang · P. Liu · J. Qiao
The Centre of Reproductive Medicine,
Peking University Third Hospital,
Beijing 100083, China
e-mail: yjianchen@hotmail.com

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However, one recent study gave conflicting results, showing a high percentage of unbalanced spermatozoa in two Rob translocation carriers [13].

More recently, interchromosomal effects (ICE) have been described for several chromosome pairs in Rob translocations. Contradictory data have been reported on the analysis of spermatozoa. Several studies have found such an ICE in male carriers of Rob translocations [8, 14–17], but others did not [12, 18, 19].

In the present work, we analyzed the meiotic segregation behavior in spermatozoa from six carriers of Rob translocations: four t(13;14), one t(14;22) and one t(13;21). Moreover, the possibility of ICE in Rob translocations was also investigated.

Materials and methods

Patients

Prior to this study, all patients signed a consent form which was approved by the ethical board of the Peking University Third Hospital.

Six unrelated men heterozygous of Rob translocations were included in this study because of infertility or repeated abortions. The somatic karyotypes were 45,XY,t(13;14)(q10;q10) in four cases (P1, P2, P3, P4), 45,XY,t(14;22)(q10;q10) in one case (P5), 45,XY,t(13;21)(q10;q10) in one case (P6). The age of the patients ranged from 30 to 38 years.

Three males with a normal karyotype and normal sperm parameters were used as controls. Their age ranged from 32 to 36 years. Their clinical history and physical examination revealed no abnormalities that could be a factor of infertility. They were involved in assisted reproductive therapy because of a female reproductive problem.

The cytogenetic and spermiologic characteristics of the patients and controls were given in Table 1.

Sperm preparation

Semen samples were washed two times in fertilization medium by centrifugation (300 g, 10 min), then dropped onto clean microscope slides and air dried, fixed for 10 min in fresh fixative (3:1, methanol:glacial acetic acid), then stored at -20°C . Before hybridization, for decondensation of the sperm nuclei, the sperm slides were first immersed in a jar of 10 mM DTT for 30 min, and then 10 mM LIS/1 mM DTT solution for 3–4 h at room temperature, subsequently rinsed with $2\times\text{SSC}$, and then passed through an ethanol series (70, 90 and 100%). After aging at 65°C for 30 min, slides were finally re-dehydrated through an ethanol series (70, 90 and 100%) and air dried.

FISH procedure

Two sets of probe mixtures were used in this study.

Firstly, for the detection of normal/balanced or unbalanced sperm, dual-color FISH was carried out using locus-specific probes (LSP) and Tel probes from Vysis (Vysis, Downers Grove, IL, USA). Probes used for each translocation carrier: t(13;14) carrier: LSI13 (13q14, Spectrum Green) and Tel14 (subtelomere 14q, Spectrum Orange); t(14;22) carrier: Tel14 (subtelomere 14q, Spectrum Orange) and LSI22 (22q12.3, Spectrum Green); t(13;21) carrier: LSI13 (13q14, Spectrum Green) and LSI21 (21q22.13–q22.2, Spectrum Orange).

Secondly, to investigate the presence of ICE, triple-color FISH was performed using the second probe mixture which consist of commercial satellite (DNA) probes from Vysis, including chromosomes 18, X and Y (CEP 18, Spectrum Aqua/CEP X, Spectrum Green/CEP Y, Spectrum Orange).

Table 1 Cytogenetic and spermiologic results of six carriers of Rob translocations and three controls

Group	Patient	Age (years)	Karyotype	Sperm concentration ($\times 10^6/\text{ml}$)	Mobility (a+b) (%)	Reproductive history
Carriers	P1	38	45,XY,t(13;14)(q10;q10)	14	23	G1P0A1
	P2	30	45,XY,t(13;14)(q10;q10)	11.2	5.27	G0P0
	P3	33	45,XY,t(13;14)(q10;q10)	0.6	1	G0P0
	P4	30	45,XY,t(13;14)(q10;q10)	1.3	3	G0P0
	P5	35	45,XY,t(14;22)(q10;q10)	291	50.55	G7P0A7
	P6	36	45,XY,t(13;21)(q10;q10)	11.7	9.88	G1P0A1
Controls	C1	32	46,XY	45	58.4	G0P0
	C2	36	46,XY	67	57.8	G0P0
	C3	34	46,XY	56	53.6	G0P0

G gestation, P parity, A abortion

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 six patients

Segregation modes	Chromosomal status	FISH results					
		P1 Count (%)	P2 Count (%)	P3 Count (%)	P4 Count (%)	P5 Count (%)	P6 Count (%)
Alternate	Normal or balanced	907 (82.76)	1,630 (81.26)	336 (78.14)	433 (83.91)	4148 (80.51)	5,200 (86.88)
Adjacent	Nullisomy A	62 (5.66)	136 (6.78)	18 (4.19)	24 (4.65)	324 (6.29)	180 (3.01)
	Disomy A	43 (3.92)	48 (2.39)*	10 (2.33)	18 (3.49)	160 (3.11)*	185 (3.09)
	Nullisomy B	39 (3.56)	100 (4.99)	46 (10.70)	20 (3.88)	252 (4.89)	155 (2.59)
	Disomy B	36 (3.28)	56 (2.79)*	10 (2.33)*	11 (2.13)	236 (4.58)	180 (3.01)
	Total	180 (16.42)	340 (16.95)	84 (19.53)	73 (14.15)	972 (18.87)	700 (11.70)
3:0 or diploid	Diploid	9 (0.82)	18 (0.90)	10 (2.33)	8 (1.55)	28 (0.54)	45 (0.75)
Other		0	18 (0.90)	0	2 (0.39)	4 (0.08)	40 (0.67)
Total		1,096	2006	430	516	5152	5985

A=first chromosome involved in the Rob translocation; B=second chromosome involved in the Rob translocation

* $P < 0.05$ disomy compared with nullisomy in each carrier

The probes were prepared according to the manufacturer's instructions. Briefly, two or three probes were mixed equally (1 μ l of each probe), and added to hybridization buffer (50% formamide/ 50% dextran sulphate) to a final volume of 10 μ l. Probes and sperm slides were denatured separately for 5 min at 75°C in a water bath. Each probe mix was applied to the denatured slides, and slides were covered with coverslips, sealed with rubber cement and hybridized overnight in a dark, moist chamber at 37°C. Coverslips were gently removed after hybridization, and the slides were washed three times in 50% formamide/2×SSC solution at 45°C, then once in 2×SSC at 45°C, and once in 2×SSC/0.1% NP-40 at 45°C and finally mounted with 10 μ l DAPI (4',6-Diamidino-2-phenylindole) in antifade

solution. The sperm slides were analyzed by two independent observers using a Nikon fluorescence microscope equipped with a filter set for FITC, Texas Red, Aqua and DAPI/Texas Red/FITC.

Sperm FISH scoring criteria

Only morphologically intact sperm were assessed according to the standard assessment criteria. Briefly, overlapping sperm nuclei, disrupted nuclei or large nuclei with diffuse signals were not considered. Sperm nuclei were scored as disomy when the two spots were of equal size and intensity and were separated by at least the diameter of one hybridization domain. The absence of signal for a single

Table 3 Incidence of sperm nullisomy, disomy and diploid for chromosomes 18, X and Y in six Rob translocation carriers and controls

	Sperm count	Norma/balanced Count (%)	Chromosome 18		Sex chromosome		Diploid Count (%)	other Count (%)
			Nullisomy Count (%)	Disomy Count (%)	Nullisomy Count (%)	Disomy Count (%)		
P1	1,046	1,032 (98.66)	3 (0.29)	0	2 (0.19)	7 (0.67)	2 (0.19)	0
P2	1,053	1,013 (96.20)	2 (0.19)	1 (0.09)	26 (2.47)*	6 (0.57)	5 (0.47)	0
P3	263	218 (82.89)	2 (0.76)	0	7 (2.66)*	12 (4.56)*	17 (6.46)*	7 (2.66)*
P4	698	670 (95.99)	1 (0.14)	0	4 (0.57)	2 (0.29)	21 (3.01)*	0
P5	2,561	2,544 (99.34)	0	0	13 (0.51)	3 (0.12)	1 (0.04)	0
P6	1,190	1,158 (97.31)	5 (0.42)	1 (0.08)	18 (1.51)*	2 (0.17)	5 (0.42)	1 (0.08)
C1	1,027	1,022 (99.51)	2 (0.19)	1 (0.1)	1 (0.1)	0	1 (0.1)	0
C2	1,073	1,069 (99.63)	1 (0.09)	0	3 (0.28)	0	0	0
C3	1,033	1,024 (99.13)	1 (0.1)	0	5 (0.48)	3 (0.29)	0	0
Control/mean	1,044	1,038 (99.43)	1 (0.1)	0.3 (0.03)	3 (0.29)	1 (0.1)	0.3 (0.03)	0

* $P < 0.05$ compared with controls

chromosome was scored as nullisomy for this chromosome only when the other probed chromosomes gave a signal.

Data analysis

The χ^2 test was used to statistically analyze the rates of nullisomy and disomy observed in patients and compare the results of aneuploidy for chromosome 18, X and Y between the translocation carriers and the control subjects. Differences were considered to be significant when $P < 0.05$.

Results

A total of 15,245 sperm nuclei from the six translocation carriers (range: 430–5,985) were analyzed for this study. The results of the segregation analysis are detailed in Table 2. The incidence of normal/balanced sperm nuclei resulting from alternate segregation ranged from 78.14 to 86.88%. The frequency of unbalanced spermatozoa resulting from adjacent segregation varied between 11.70 and 19.53%. The rate for 3:0 segregations and diploid spermatozoa, indicated by two signals for each of translocation chromosome, ranged from 0.54 to 2.33%. Spermatozoa with an unexpected combination of signals according to the theoretical segregations were classified as “others” and ranged between 0 and 0.90%.

In five patients (P1, P2, P3, P4, P5), the incidences of nullisomy for each chromosome involved in the translocation were higher than the complementary frequencies of disomy. The differences reached a significant level in patient 2 for the nullisomy 13 and 14 rates and in patient 3 and 5 for the nullisomy 14 rate ($P < 0.05\%$). In patient 6, the incidences of disomy for both chromosomes were higher than the complementary frequencies of nullisomy, but no significant difference ($P > 0.05$) (Table 2).

The total number of spermatozoa scored, and the nullisomy, disomy and diploid rates for chromosomes 18, X and Y in six Rob translocation carriers and controls are summarized in Table 3. In patients 2, 3 and 6, the incidence of spermatozoa with nullisomy for the sex chromosomes was significantly higher compared to that of controls ($P < 0.05$). The rate of spermatozoa with disomy for the sex chromosome was significantly higher in patient 3. In addition, the rate of diploid was significantly higher in patient 3 and 4 compared with controls ($P < 0.05$).

Discussion

The present work demonstrated that the incidence of normal/balanced spermatozoa resulting from alternate segregation ranged from 78.14 to 86.88% in six male carriers

of Rob translocations. The meiotic segregation behavior showed a high predominance of alternate segregation in spermatozoa from common and uncommon Rob translocation carriers. This finding is consistent with previous published data [7–9, 11, 12, 17, 20]. The high prevalence of the alternate segregation had been presumed to occur because cis-configuration of the trivalent during meiosis favors an alternate segregation in all Rob translocations [21–23].

T(13;14) is the most frequency kind of Rob translocation. Among the previously most well-studied t(13;14) cases, normal or balanced spermatozoa were predominant and ranged from 73.5 to 92.3%, the rate of unbalanced spermatozoa ranged from 7.70 to 26.5% [8, 17]. However, recently one paper reported the mean rate of unbalanced spermatozoa resulting from the adjacent segregation reached 43.58% in two t(13;14) translocation carriers [13]. In the present manuscript, the rates of unbalanced spermatozoa range from 14.15 to 19.53% in four t(13;14) carriers. This discrepancy may be due to interindividual variabilities. Another reason could have been the technical aspects of sperm FISH studies, such as the decondensation and the denaturation of sperm nuclei and the hybridization efficiency of the different types of probe [17, 24].

In the group of the four t(13;14) carriers, the nullisomy rates for chromosomes 13 and 14 were higher than that of disomy. Similar results were reported previously by other researchers [6, 17, 25, 26], but this phenomenon remain to be further investigated.

The t(14;22) and t(13;21) are two rare Rob translocations, the meiotic segregation pattern in spermatozoa only have two and one reports available in the literature by now, respectively [11, 12, 27]. Our data showed that alternate segregation was largely dominant over adjacent segregations in the two uncommon Rob translocations carriers (patient 5 and patient 6). This finding is consistent with results from previous studies of rare Rob translocations t(14;22) and t(13;21). However, there was a difference in the rates of nullisomy and disomy between our data and the published data. A higher nullisomy rate for chromosome 14 and 22 were observed in the patient 5, whereas Moradkhani [11] reported the disomy rates for chromosome 14 and 22 were always higher than the nullisomy rates in three carriers with t(14;22). For both chromosomes 13 and 21, the disomy rates were higher than that of nullisomy in the patient 6, in contrast, the reduced incidences of disomy over nullisomy were reported in another study [12]. Due to limited literature data in uncommon Rob translocation, meiotic segregation analysis of more cases of the rare Rob translocations are required to accurately investigate this question.

ICE first described by Lejeune [28], but the hypothesis of an ICE associated with carriers of Rob translocations has

always been a controversial issue. Some reports supported the possibility of ICE in Rob translocations [5, 8, 14–17, 26]. However, others demonstrated no evidence of this phenomenon [12, 18, 19].

In the present study, the significant increased rates of nullisomy for the sex chromosomes were observed in two t(13;14) carriers and one t(13;21) carrier (P2, P3 and P6). In patient 3, the increased rate of disomy for the sex chromosome was found. In addition, the rates of diploid were significantly higher in two t(13;14) carriers (P3 and P4) compared with controls. Similar results were made previously by other studies [15–17]. This mechanism of meiotic non-disjunction for sex chromosomes may be the results of a non-random association at prophase I between the trivalent and the sex chromosomes [22, 23].

More interesting, the occurrence of an ICE seems to be related to sperm quality, but may be independent of the type of Rob translocation. In current study, the possibility of ICE was found in common and uncommon Rob translocations with poor sperm quality (P2, P3, P4 and P6). Reviewing the published data, Rob translocation carriers with an increased rate of aneuploidy always had the oligozoospermia or oligoasthenoteratozoospermia [15–17]. Therefore, more cases should be investigated to confirm whether the increased aneuploidy frequency in Rob translocation carriers is truly the result of ICE or is associated with abnormal sperm.

In conclusion, this present work supports that alternate segregation is largely dominant over adjacent segregations in spermatozoa from the different types of Rob translocation carriers. Furthermore, the higher incidences of aneuploidy for sex chromosomes in spermatozoa were found in three Rob translocation carriers, which indicated that the ICE on sex chromosome is likely in some male carriers of Rob translocations.

Taken altogether, our study suggests that genetic counseling is important for the carriers of Rob translocations. In order to maximize the chances of normal pregnancy, we highly recommend that normal or balanced embryo should be selected for transfer by preimplantation genetic diagnosis analysis of translocation chromosome, and combining with a preimplantation genetic screening for sex chromosome aneuploidy.

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