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Chromosomal segregation in sperm of Robertsonian translocation carriers

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Abstract
Purpose To study meiotic segregation patterns of Robertsonian translocations in sperm of male carriers and to assess the frequencies of unbalanced sperm formation.
Methods FISH with combination of probes to detect all the variants of meiotic segregation was performed on decondensed sperm nuclei of 5 carriers of der(13;14), 3 carriers of der(14;21) and one carrier of a rare der(13;21) translocation.
Results The frequency of sperm with alternate segregation and normal/balanced chromosomal complement ranged from 68 % to 94.4 % (mean 79.2±8.4). Adjacent segregation was detected in 17.9±7.3 % of sperm (from 5.6 % to 29 %). No significant differences in frequencies of gametes with nullisomies and disomies of chromosomes involved in translocations were observed. The mean frequency of 3:0 segregation products was 2.5±1.4 %.
Conclusions All analyzed patients showed homogenous segregation pattern with clear predominance of alternate segregation resulting in normal/balanced sperm production. Still, from 5.8–32 % (mean 20.4±8.3 %) of sperm was unbalanced, which is the evidence of the increased risk of unbalanced offspring in carriers of Robertsonian translocations. Our results highlight the importance of genetic counseling of Robertsonian translocation carriers prior to ICSI or IVF.

Keywords Robertsonian translocation · Meiotic segregation · Unbalanced sperm · FISH

Capsule Results of meiotic segregation studies by FISH in sperm of 9 carriers of Robertsonian translocations are presented. Despite the predominant production of normal/balanced sperm a relatively high percentage of unbalanced sperm (mean 20.4±8.3 %) was detected warranting genetic counseling for Robertsonian translocation carriers before ICSI or IVF

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Introduction

Robertsonian translocations are the most common structural chromosomal rearrangements observed with an incidence of 1.23 per thousand newborns [23], with almost a 9-fold increase in groups of infertile men [6]. The majority of Robertsonian translocations involves two non-homologous chromosomes and occurs between chromosomes 13 and 14 or chromosomes 14 and 21 (73 % and 10 % of all Robertsonian translocations, respectively). Other possible combinations are less frequent. Der(13;21) is one of the most rare Robertsonian translocations, constituting approximately 2 % of all detected Robertsonian translocations [32].

Carriers of Robertsonian translocations are phenotypically normal. However, men usually have spermatogenic disruptions expressed by decreased sperm count, motility and altered morphology. Apart from reduced reproductive potential, carriers also have increased risk of miscarriage and livebirth of children with chromosomal abnormalities due to the production of unbalanced gametes. In Robertsonian translocations, pairing of the derivative chromosomes and two normal homologues in prophase I gives rise to a trivalent structure [18, 33], which can segregate in different ways at anaphase. Only products of alternate segregation have normal/balanced karyotype. All other segregation modes (adjacent-1, adjacent-2, 3:0) produce unbalanced gametes with disomies and nullisomies of chromosomes involved in Robertsonian translocations. It is well known that meiotic tetravalent configuration tends to segregate in alternate way

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Table 1 Semen parameters and reproductive history of Robertsonian translocation carriers

#	Karyotype	Sperm count, $\times 10^6$/ml	Abnormal sperm parameters	Reproductive history
1	der(13;14)	58	AT	G_1P_1
2	der(13;14)	215	A	$G_2P_0A_2$
3	der(13;14)	4	OAT	$G_1P_0A_1$
4	der(13;14)	5	OAT	G_0P_0
5	der(13;14)	8	OAT	G_0P_0
6	der(14;21)	2	OAT	G_0P_0
7	der(14;21)	4	OAT	$G_2P_0A_2$
8	der(14;21)	58	AT	G_0P_0
9	der(13;21)	65	AT	G_0P_0

A asthenozoospermia, *AT* asthenoteratozoospermia, *OAT* oligoasthenoteratozoospermia, *G* gestation, *P* parity, *A* abortion

[31], resulting in preferential production of normal/balanced spermatozoa. However, certain percentages of unbalanced gametes derived from adjacent segregation are also produced, leading to the increased risk of miscarriage and pregnancy with or livebirth of chromosomally unbalanced fetus. The potentially liveborn chromosomally unbalanced outcome of the most common der(13;14) translocation is trisomy 13 (Patau syndrome) with an empirical risk of occurrence at second trimester prenatal diagnosis of 0.4 %. There is also 0.6–0.8 % risk of uniparental disomy of chromosome 14 in carriers of der(13;14) [30]. For female carriers of der(14;21), the estimated risk of trisomy 21 at second trimester prenatal diagnosis is 15 %, whereas for male carriers this risk is below 0.5 % [16]. Since other Robertsonian translocations are less common, specific risks have not been well established. Therefore, carriers of Robertsonian translocations have two reproductive options: spontaneous pregnancy followed by prenatal diagnosis or assisted reproduction with preimplantation genetic diagnosis (PGD). According to earlier studies, PGD improves the livebirth rate by reducing the risk of recurrent miscarriages, decreasing the risk of conceiving a chromosomally abnormal child and improving the pregnancy rates in Robertsonian translocation carriers [10, 22]. The amount of unbalanced sperm in each specific case can be predictive of the proportion of unbalances embryos in PGD cycles. Therefore, in male carriers of Robertsonian translocations cytogenetic analysis of sperm nuclei by fluorescence in situ hybridization (FISH) technique with chromosome-specific probes can be performed to estimate the amount of unbalanced sperm in each translocation carrier and personalize reproductive risks.

Here we report results of chromosome segregation studies in sperm of 9 carriers of Robertsonian translocations and provide an overview of data already reported in the literature.

Materials and methods

Patients

9 unrelated men were included in the study. Somatic karyotypes were 45,XY,der(13;14)(q10;q10) in 5 men, 45,XY, der(14;21)(q10;q10) in 3 men, 45,XY,der(13;21)(q10;q10) in one man. Semen parameters and reproductive history of

Table 2 Results of meiotic segregation analysis in sperm of Robertsonian translocation carriers

Patient no.	Alt, %	Adj-1, %,	Adj-1, %	Adj-2, %	Adj-2, %	3:0, %	Σ unbalanced, %
der(13;14)							
	normal or balanced	nullisomy 14	disomy 14	nullisomy 13	disomy 13	3:0	unbalanced
1	78.4	5.4	3.2	6.9	4.8	1.4	21.7
2	75.5	5.3	4.1	4.4	6.1	2.0	21.9
3	81.2	4.2	3.8	3.6	2.9	3.7	18.2
4	86.5	3.2	3.1	1.7	4.3	1.1	13.4
5	69.4	8.6	7.3	4.4	5.9	4.2	30.4
der(14;21)							
	normal or balanced	nullisomy 21	disomy 21	nullisomy 14	disomy 14	3:0	unbalanced
6	75.2	4.3	6.2	4.4	5.8	3.8	24.8
7	84.3	4.5	3.3	2.1	2.9	2.8	15.6
8	68.0	6.3	7.4	7.5	7.8	3.0	32.0
der(13;21)							
	normal or balanced	nullisomy 21	disomy 21	nullisomy 13	disomy 13	3:0	unbalanced
9	94.4	1.3	1.5	1.1	1.7	0.2	5.8
Mean ± SD	79.2±8.4	4.8±2.0	4.4±2.1	4.0±2.2	4.7±1.9	2.5±1.4	20.4±8.3

patients are presented in Table 1. The age of patients ranged from 26 to 36 (mean 31.4±4.5).

Sperm preparation, FISH and scoring

All semen samples were first analyzed to evaluate volume, concentration and motility, according to the World Health Organization criteria [35]; morphology was assessed according to strict criteria [17]. After semen analysis, sperm with progressive motility was isolated and washed twice in Phosphate Buffered Saline (pH 7.4) by centrifugation at 400 g for 10 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 %, 80 %, 96 %), denaturation was performed in 0.25 % formamide in 2xSSC followed by overnight hybridization with a combination of commercially available probes (LSI 13q14.3 (SpectrumOrange), Vysis and 14qter subtelomere specific probe (SpectrumGreen), Cytocell for der(13;14) carriers; TelVysion21q (SpectrumOrange), Vysis and 14qter subtelomere specific probe (SpectrumGreen), Cytocell for der(14;21) carriers; LSI 13q34 (SpectrumGreen) and TelVysion21q (SpectrumOrange), Vysis for der(13;21) carrier). Post-hybridization washes included 2 min in 0.4xSSC/0.3%NP-40 (pH=7) at 72 °C, followed by 1 min in 2xSSC/0.1%NP-40 (pH=7) at room temperature. Slides were covered with DAPI II (Vysis). Soring was performed by two independent observers. 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 % and more were analyzed. 1,000 sperm nuclei per patient were analyzed.

Statistical analysis

Chi-squared test was used to compare frequencies of segregation products. A probability value of less than 0.05 was considered to be statistically significant.

Results and discussion

Multicolor FISH on decondensed sperm nuclei allows for a rapid analysis of meiotic segregation in sperm of translocation carriers, providing information on the exact amount of normal/balanced sperm. Accumulation of such information is undoubtedly important not only for basic cytogenetic research but also for reproductive counseling of Robertsonian translocation carriers. Data obtained in the current study show the predominance of alternate segregation with the preferential production of normal/balanced sperm in carriers of Robertsonian translocations. The observed frequency of normal/balanced sperm in the studied group ranged from 68 % to 94.4 % (mean 79.2±8.4) (Table 2). No significant difference in mean frequencies of normal/balanced sperm depending on translocation type was observed. Our results are consistent with earlier published data, providing evidence that alternate segregation is predominant in sperm of Robertsonian translocation carriers, irrespective of the technique used in the study (hamster egg-human sperm fusion, sperm injection into mouse oocytes or FISH) (Table 3). It should be mentioned, however, that the range of frequencies of normal/balanced gametes in our group (from 68–94.4 %) is wider, than

Table 3 Review of literature on meiotic segregation of chromosomes in sperm of Robertsonian translocation carriers

Reference	Number of patients	Number of cells	Mean frequency of sperm with alternate segregation, %
der(13;14)			
[26][a]	1	78	92.3
[20][a]	1	117	73.5
[24][b]	1	45	91.1
[8]	2	2,022	75.5
[11]	3	3,116	89.4
[21]	3	5,102	84.3
[25]	7	5,213	84.6
[15]	1	1,629	90.9
[27]	6	8,775	86.1
[5]	2	600	84.9
[9]	11	9,881	81.8
[19]	5	842	71.5
[28]	1	2,000	80.7
Present study	5	5,000	78.2
der(14;21)			
[1][a]	1	24	87.5
[29]	1	1,116	72.2
[13]	1	16,578	88.4
[11]	3	3,000	92.4
[25]	2	2,098	86.8
[4]	1	4,602	85.9
[9]	8	8,515	90.3
Present study	3	3,000	75.8
der(13;21)			
[12]	1	10,223	88.4
[7]	1	5,152	86.9
[3]	1	3,022	86.3
Present study	1	1,000	94.4

[a] studies performed by the hamster egg-human sperm fusion heterospecific fertilization

[b] study by sperm injection into mouse oocytes

reported in earlier published studies (from 71.5–92.4 %). Variability in frequencies of unbalanced sperm in different studies can be related to technical aspects, such as FISH protocols, probes or scoring criteria used. Still, certain amount of inter-individual differences in samples studied in the same laboratory can be caused by specific patients' characteristics, such as seminal parameters or infertility history. We did not detect a clear association between reduced sperm count and unbalanced sperm complement, since mean frequencies of unbalanced sperm in oligozoospermic group and group of patients with sperm count above 20×10^6/ml did not differ significantly (20.9 % vs 20.4 %; $p=0.9$). Such an association, however, was observed by Ferfouri and colleagues [9] in a group of 29 Robertsonian translocation carriers. Authors of several studies attempted to identify other factors, which may correlate with chromosomal complement. To date, no association of sperm maturation, morphology and motility with the amount of unbalanced sperm was found [5, 34]. Therefore, the use of high magnification sperm selection or hyaluronan binding assays for selection of sperm with normal/balanced chromosomal complement is not effective [5, 34]. Taking into consideration relatively high levels of unbalanced sperm (from 5.8–32 %) and unbalanced embryos (as reported in results of PGD cycles [2, 14]), PGD is justified for Robertsonian translocation carriers to reduce the risk of miscarriage and to increase the chances of pregnancy achievement.

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