

Chromosomal segregation in spermatozoa of 14 Robertsonian translocation carriers

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Male carriers of Robertsonian (Rob) translocations can have fertility problems associated with low sperm counts and abnormal sperm morphology. In this study, spermatozoa from 14 Rob translocation carriers, seven der(13;14), two der(13;15), two der(14;15), two der(14;21) and one der(21;22), were tested by fluorescence in-situ hybridization (FISH) for the chromosomes involved, to study meiotic segregation behaviour. It was shown that in each type of Rob translocation, meiotic segregation behaviour is similar, comparable and occurs non-randomly. Most of the spermatozoa results from alternate segregation (range: 76–89.47%). There is, however, still much unbalanced spermatozoa resulting from adjacent segregation mode (range: 10.24–23.41%). These data provide useful information for genetic counselling purposes. Moreover, aneuploidy for chromosomes 13,18, 21, X and Y was studied in five patients and suggested an inter-chromosomal effect.

Key words: chromosomes/FISH/Robertsonian translocation/sperm segregation

Introduction

Robertsonian (Rob) translocations are one of the most common structural chromosomal aberrations observed in humans with an incidence of 1.23 per thousand births (Nielsen and Wohlert, 1991). These special types of translocations originate through centric fusion of the long arms of the acrocentric chromosomes 13, 14, 15, 21 and 22, with the translocated chromosome bearing either one or two centromeres. Usually, a simultaneous loss of both short arms is observed (ISCN, 1995; Gardner and Sutherland, 1996). The most frequent Rob translocations are der(13;14) (0.97 per thousand), followed by der(14;21) (0.20 per thousand) (Nielsen and Wohlert, 1991). In general, carriers of Rob translocations are phenotypically normal but are at increased risk for spontaneous abortions and chromosomally unbalanced offspring. In addition, male carriers of Rob translocations do have more frequent fertility problems because oligoasthenoteratozoospermia (OAT). A review of the largest and most relevant series of karyotypes in oligozoospermic males shows that carriers of Rob translocations occur in 1.6% of the patients. This is 13 times higher than the frequency reported in the newborn study of Nielsen and Wohlert (1991) (for review, see Van Assche *et al.*, 1996).

Cytogenetic analysis of sperm nuclei using fluorescence in-situ hybridization (FISH) techniques with different chromosome-specific probes has proven to be a useful tool to determine the meiotic segregation behaviour especially in translocation carriers. Analysing cells by using FISH is less time-consuming than the hamster ovum penetration test and karyotyping (Balkan and Martin, 1983; Pellestor *et al.*, 1987; Martin, 1988; Syme and Martin, 1992), and many cells can be examined in a relatively short period (Rousseaux *et al.*, 1995; Escudero *et al.*, 2000; Honda *et al.*, 2000; Frydman *et al.*, 2001; Morel *et al.*,

2001; Anton *et al.*, 2004). In addition, the application of FISH analysis, using probes for additional chromosomes, allows examination of the possible existence of inter-chromosomal effects.

In this study, the meiotic segregation behaviour in spermatozoa from 14 Rob translocation carriers with abnormal sperm parameters were determined by FISH. The different segregation modes of 14 patients and the inter-individual variation was investigated. Moreover, the different patterns of segregation between the specific translocation types were compared to verify the existence of a preferential mode of segregation. Inter-chromosomal effects of a Rob translocation on chromosomes 13, 18, 21, X and Y were also investigated in spermatozoa from five carriers: one der(13;14), one der(13;15), two der(14;15) and one der(14;21) carrier. Finally, an overview of data already reported in the literature is provided and set against our own data for comparison and discussion.

Materials and methods

Patients

Fourteen unrelated male Rob translocation carriers were included in the study. The somatic karyotypes were 45,XY,der(13;14)(q10;q10) in seven males; 45,XY,der(13;15)(q10;q10) in two males; 45,XY,der(14;15)(q10;q10) in two males; 45,XY,der(14;21)(q10;q10) in two males and 45,XY,der(21;22)(q10;q10) in one male. The semen concentration was abnormal for 12 males and ranged between 0.13 and 12.9 × 10⁶ spermatozoa/ml. Their sperm motility and morphology were below the normal level (see Table I). In two males, the sperm concentration was normal (22.4 and 26.5 × 10⁶ spermatozoa/ml in patient 12 and 8, respectively), but the other parameters were disturbed. The age of the patients ranged from 27 to 45 years. Nine of them were infertile, whereas the other five had achieved a total of six pregnancies of which five miscarried and one led to the birth of a healthy child (Table I).

Table I. Semen parameters and reproductive histories of 14 Robertsonian (Rob) translocation carriers

Type of Rob translocation	Semen concentration $\times 10^6$ /ml (abnormal sperm parameters)*	Reproductive history
der(13;14)	2.60 (OAT)	G ₂ P ₀ A ₂ one IVF failure
	1.50 (OAT)	G ₀ P ₀ A ₀
	12.90 (OAT)	G ₀ P ₀ A ₀ one ICSI failure
	0.50 (OAT)	G ₀ P ₀ A ₀
	4.70 (OAT)	G ₁ P ₀ A ₁ five IVF failures
	2.20 (OAT)	G ₁ P ₀ A ₁
der(13;15)	0.13 (OAT)	G ₀ P ₀ A ₀ four IVF failures
	26.50 (AT)	G ₁ P ₁ A ₀
der(14;15)	5.80 (OAT)	G ₀ P ₀ A ₀
	0.25 (OAT)	G ₁ P ₀ A ₁
der(14;21)	1.68 (OAT)	G ₀ P ₀ A ₀
	22.40 (AT)	G ₀ P ₀ A ₀
der(21;22)	2.90 (OAT)	G ₀ P ₀ A ₀
	0.88 (OAT)	G ₀ P ₀ A ₀

A = abortion; G = gestation; OAT = oligoasthenoatozoospermia; P = parity. *World Health Organization (1999) parameters were used for concentration and motility; Kruger *et al.* (1986) for morphology.

For each different type of Rob translocation, spermatozoa of a normal control individual (=normal sperm parameters and a normal somatic karyotype) were analysed. In all, sperm analysis of five different controls was performed.

To verify a possible inter-chromosomal effect of the Rob translocation on chromosomes 13, 18, 21, X and Y, we compared our results, obtained in spermatozoa of one der(13;14), one der(13;15), two der(14;15) and one der(14;21) carrier, with those of 13 healthy controls with normal peripheral blood karyotype and normal semen parameters (same control group as used by Vegetti *et al.*, 2000).

Sperm preparation

All the semen samples were first analysed to evaluate volume, concentration and motility, according to the World Health Organization (1999) parameters, and morphology, according to strict criteria (Kruger *et al.*, 1986). Semen samples with volume ≥ 2 ml, with sperm concentration $\geq 20 \times 10^6$ /ml, percentage of spermatozoa with total progressive motility $\geq 50\%$ and with normal morphology $\geq 14\%$ were regarded as normal. The sperm samples were washed three times in phosphate-buffered saline (PBS), pH 7.2, and centrifuged at 280 g for 10 min, and the sediment was then fixed in methanol/acetic acid (3:1). After three rinses with fresh fixative, the fixed spermatozoa were spread onto Superfrost Plus glass slides (Kindler, Freiburg, Germany). At least two slides were prepared for each patient. The slides were stored at -20°C until further processing. For decondensation of the sperm nuclei, slides were first rinsed briefly in 2 \times standard saline citrate (SSC) solution, immersed in 25 mmol/l dithiothreitol (DTT; Sigma, St. Louis, MO, USA) in a 0.1 M Tris-HCl solution (pH 8.0) for 5 min and subsequently rinsed again in 2 \times SSC and finally dehydrated through an ethanol series and air-dried (Vegetti *et al.*, 2000).

FISH procedure, microscopy and scoring criteria

Dual- and triple-colour in-situ hybridization analysis using direct-labelled subtelomeric and/or locus-specific and/or centromeric probes for chromosomes 13, 14, 15, 21 and 22 was carried out on spermatozoa of the translocation carriers. In three random cases, a centromere-specific probe for chromosome 10 was used as an internal control to make the differentiation of 3:0 segregation possible (for details of the probe mixtures, that were used, see Table II). Spermatozoa from five males [patient 3 = carrier of der(13;14), patient 10 and 11 = carrier of der(14;15), patient 8 = carrier of der(13;15) and patient 13 = carrier of der(14;21)] were also screened for the enumeration of chromosomes 13, 18, 21, X and Y. Two Vysis® (Downers Grove, IL, USA) probe mixtures were used: probe 13 (LSI® 13q14 spectrum green) and probe 21 (LSI® 21q22.13–21q22.2 spectrum orange) (=mixture 1) and probe 18 (CEP® a sat D18Z1 spectrum aqua), probe X (CEP® a sat DXZ1 spectrum green) and probe Y (CEP® a sat DYZ3 spectrum orange) (=mixture 2). The concentration of the different

Table II. Probes used for each translocation carrier (mixture 1)

Rob translocation	Probe mixtures	
der(13;14) Patients 1–6 Patient 7	LSI® 13q14 SG/subtelomere 14q R CEP® α sat D10Z1 SA LSI® 13q14 SG Subtelomere 14q R	
	der(13;15) Patient 8	CEP® α sat D10Z1 SA Subtelomere 13q R Subtelomere 15q G Subtelomere 13q R/subtelomere 15q G
		Patient 9 der(14;15) Patient 10
Patient 11 der(14;21) Patient 12 Patient 13	LSI® 21q22.13–21q22.2 SO/subtelomere 14q G CEP® α sat D10Z1 SA LSI® 21q22.13–21q22.2 SO Subtelomere 14q G	
	der(21;22) Patient 14	LSI® 21q22.13–21q22.2 SO/subtelomere 22q G

Probes labelled with spectrum aqua (SA), spectrum green (SG) and spectrum orange (SO) (Vysis®). Probes labelled with red or green (Cytocell, Cambridge, UK).

probes was 1.2 μl for the region-specific probes, 1.0 μl for the centromeric probes and 2.5–3.0 μl for the sub-telomeric probes. For each probe mixture, hybridization buffer was added until a final volume of 10 μl .

The FISH procedure was applied, as described previously (Vegetti *et al.*, 2000). FISH signals on the slides were evaluated using a Zeiss-Axioplan fluorescence microscope with the appropriate filter sets (Vysis®). An automated chromosome analysis and capturing system of Applied Imaging (Cytovision) was used. Only slides with a hybridization rate $\geq 98\%$ were analysed, and at least 500 sperm nuclei per probe mixture were scored.

Stringent scoring criteria of Williams *et al.* (1993) were used. Only intact and well-delineated spermatozoa were used for FISH signal scoring. We considered only clear hybridization signals, similar in size, separated from each other by at least one signal domain and clearly positioned within the sperm head (Williams *et al.*, 1993; Vegetti *et al.*, 2000). Spermatozoa were regarded as disomic if they presented two distinct hybridization signals for the same chromosome, each equal in intensity and size to the single signal found in normal monosomic nuclei.

Statistical analysis

Chi-squared analysis was used to compare the frequencies of adjacent and alternate segregation patterns observed in the specific translocation groups with those in the control population. Each patient in each group was compared with the control in that group. In addition, the results obtained for each translocation group were compared (chi-squared analysis and multiple testing correction). A probability value of less than 0.05 was considered to be statistically significant.

Results

Between 500 and 2663 spermatozoa were analysed per patient or control, leading to 18 150 spermatozoa, 12 824 in the Rob translocation carriers and 5326 in the controls. The number of spermatozoa scored, the alternate mode of segregation, nullisomy, disomy and diploidy rates for each patient and each control are summarized in Table III. Overall, the frequency of balanced spermatozoa resulting from alternate segregation varied between 76 and 89.47% per patient. The rates

Table III. Incidence of sperm nullisomy, disomy and diploidy for the chromosomes involved in the Robertsonian (Rob) translocation in 14 patients and five controls

Type of Rob translocation	Patient	Sperm count	Alternate sperm count (%)	Chromosome A (sperm count)			Chromosome B (sperm count)			Adjacent (A + B) sperm count (%)	Other (%)	3:0 (%)	Diploidy (%)
				Nullisomy	Disomy	Total	Nullisomy	Disomy	Total				
der(13;14)													
	1	500	405 (81.81)	23	24	47	26	20	46	93 (18.60)	0.00	0.40	
	2	500	392 (78.40)	34	25	59	27	20	47	106 (21.20)	0.00	0.40	
	3	1032	882 (85.47)	39	44	83	35	32	67	150 (14.33)	0.00	0.00	
	4	507	435 (85.80)	9	24	33	19	17	36	69 (13.67)	0.00	0.59	
	5	1001	836 (83.52)	38	41	79	44	42	86	165 (16.48)	0.00	0.00	
	6	1016	909 (89.47)	27	20	47	39	18	57	104 (10.24)	0.00	0.59	
	7	657	554 (84.32)	39	15	54	28	17	45	99 (15.07)	0.00	0.15	
Total	7 cases	5213	4413 (84.65)	209*	183*	402*	218*	166*	384*	786 (15.08)*	0.00	0.02	
46,XY	Control 1	523	518 (99.04)	2	2	4	1	0	1	5 (0.95)	0.00	0.00	
der(13;15)													
	8	1109	917 (82.69)	57	77	134	23	32	55	189 (17.04)	0.09	0.00	
	9	1021	776 (76.00)	71	28	99	89	51	140	239 (23.41)	0.00	0.59	
Total	2 cases	2130	1693 (79.48)	128*	105*	233*	112*	83*	195*	428 (20.09)*	0.05	0.38	
46,XY	Control 2	640	633 (98.91)	2	1	3	1	3	4	7 (1.09)	0.00	0.00	
der(14;15)													
	10	819	668 (81.56)	35	18	53	34	42	76	129 (15.75)	0.73	1.95	
	11	1548	1338 (86.43)	63	43	106	50	42	92	198 (12.79)	0.19	0.58	
Total	2 cases	2367	2006 (84.75)	98*	61*	159*	84*	84*	168*	327 (13.81)*	0.38	1.06	
46,XY	Control 3	500	495 (99.00)	1	1	2	1	2	3	5 (1.00)	0.00	0.00	
der(14;21)													
	12	996	875 (87.85)	25	33	58	20	40	60	118 (11.85)	0.30	0.00	
	13	1102	944 (85.66)	37	28	65	36	40	76	141 (12.79)	0.27	0.00	
Total	2 cases	2098	1819 (86.70)	62*	61*	123*	56*	80*	136*	259 (12.35)*	0.29	0.67	
46,XY	Control 4	1000	976 (97.60)	4	4	8	8	8	16	24 (2.40)	0.00	0.00	
der(21;22)													
	14	1016	870 (85.63)	33	36	69	40	26	66	135 (13.29)	0.39	0.69	
Total	1 case	1016	870 (85.63)	33*	36*	69*	40*	26*	66*	135 (13.29)*	0.39	0.69	
46,XY	Control 5	2663	2632 (98.84)	6	6	12	8	11	19	31 (1.16)	0.00	0.00	
Total	14 cases	12 824	10 801 (84.22)	530*	456*	986*	510*	439*	949*	1935 (15.08)*	0.16	0.52	
Total	5 controls	5326	5254 (98.65)	15	14	29	19	24	43	72 (1.35)	0.00	0.00	

Chromosome A, first chromosome involved in the Rob translocation; chromosome B, second chromosome involved in the Rob translocation.

* $P < 0.001$, each translocation group compared with controls.

of unbalanced spermatozoa resulting from adjacent segregation varied between 10.24 and 23.41%. By using two-colour FISH, it was not possible to differentiate between 3:0 segregations and diploid spermatozoa (both cases show two hybridization signals for the probes used). Unbalanced spermatozoa bearing this combination of signals accounted for 0–1.95%. For 12 of 14 males, the rate was below 1%. In three randomly chosen translocation carriers (patient 7 with der(13;14); patient 8 with der(13;15) and patient 13 with der(14;21), a third probe specific for an autosome not involved in the translocation and labelled with a different fluorochrome (=centromeric probe 10 labelled with spectrum aqua; Table II) was added to their respective probe mixtures to allow differentiation between diploidy and 3:0 segregation. In patients 7, 8 and 13, the rate of diploid gametes was higher than the rate of gametes resulting from 3:0 segregation: 0.46, 0.18 and 1.27% versus 0.15, 0 and 0%, respectively. Spermatozoa with an unexpected combination of signals according to the theoretical segregations were classified as 'others' and ranged between 0 and 0.73%.

In the control samples, between 97.60 and 99.05% of the spermatozoa showed a normal haploid pattern (mean: 98.65%). Between 0.96 and 2.40% of the spermatozoa were found to be nullisomic or disomic for the investigated chromosomes (mean: 1.35%). No diploid sperm cells were observed in the sperm samples of the controls.

Both the disomies and the nullisomies as well as the sum of both for each carrier and for each chromosome involved in the translocation were systematically significantly higher than in the controls ($P < 0.001$) (Table III). For the majority of different carriers, the rates of unbalanced

spermatozoa, resulting from the adjacent modes of segregation (nullisomy and disomy), were comparable. Within groups, no statistically significant differences were seen between the different patients except for patient 6 in group 1 and patient 8 and 9 in group 2 (Table III). The rates of unbalanced spermatozoa, resulting from the adjacent modes of segregation between the different Rob translocation groups were comparable. Except for group 2, no statistical difference was observed between the specific translocation groups. Furthermore within the groups, no statistical difference was observed for the rate of adjacent segregation for each chromosome involved in the translocation (Table IV).

The total number of spermatozoa scored, and the nullisomy, disomy and diploidy rates for the chromosomes 13, 18, 21, X and Y for five Rob translocation carriers and controls are reported in Tables V and VI. In all, 14 050 spermatozoa were analysed (range: 415–2473). In both carriers of der(14;15) (patients 10 and 11), the incidence of spermatozoa with disomy of the sex chromosomes was significantly higher compared with controls. In addition, diploidy for 13 and 21 in patient 10 and for 18, X and Y in patient 11 was significantly higher. In patient 3, nullisomy of the sex chromosomes was significantly higher.

Discussion

Multicolour FISH on interphase spermatozoa of translocation carriers allows for the direct analysis of segregation patterns in a high number

Table IV. The rate of adjacent segregation (nullisomy + disomy) for each chromosome involved in the Robertsonian (Rob) translocation

Patient group	Sperm count (total)	Chromosome A (nullisomy + disomy) (sperm count)	Chromosome B (nullisomy + disomy) (sperm count)	<i>P</i> *
1 [der(13;14) 7 cases]	5213	402	384	0.544
2 [der(13;15) 2 cases]	2130	233	195	0.074
3 [der(14;15) 2 cases]	2367	161	168	0.484
4 [der(14;21) 2 cases]	2098	123	136	0.456
5 [der(21;22) 1 cases]	1016	69	66	0.863

Chromosome A, first chromosome involved in the Rob translocation; chromosome B, second chromosome involved in the Rob translocation.

**P* > 0.05, chromosome A compared with chromosome B.

Table V. Incidence of sperm nullisomy, disomy and diploidy for chromosomes 13 and 21 (values are expressed in %) in five Robertsonian (Rob) translocation carriers and controls (Vegetti *et al.*, 2000)

Karyotype	Spermatozoa counted	Chromosome 13		Chromosome 21		Diploidy
		Nullisomy	Disomy	Nullisomy	Disomy	
der(13;14) (patient 3)	1009	5.00*	3.60*	0.20	0.10	0.00
der(13;15) (patient 8)	2473	10.59*	5.94*	0.36	0.20	0.20
der(14;15) (patient 10)	415	0.96	0.00	1.44	0.00	6.26†
der(14;15) (patient 11)	1006	0.89	1.09	0.79	0.99	0.69
der(14;21) (patient 13)	2342	0.59	0.34	3.50*	2.90*	0.29
Controls/mean (Vegetti <i>et al.</i> , 2000)	4442	0.13	0.08	0.11	0.07	0.03

*Not included in the statistical evaluation: the analysed chromosomes are involved in the translocation.

†*P* < 0.001.

Table VI. Incidence of sperm nullisomy, disomy and diploidy for chromosomes 18, X and Y (values are expressed in %) in five Robertsonian (Rob) translocation carriers and controls (Vegetti *et al.*, 2000)

Karyotype	Spermatozoa counted	Chromosome 18		Sex chromosomes		Diploidy
		Nullisomy	Disomy	Nullisomy	Disomy (XX + YY + XY)	
der(13;14) (patient 3)	1002	0.40	0.00	2.90*	0.30	0.00
der(13;15) (patient 8)	2167	0.59	0.22	0.36	0.27	0.09
der(14;15) (patient 10)	449	0.44	0.89	0.89	2.89*	0.44
der(14;15) (patient 11)	1023	0.29	0.97	0.87	1.75*	1.36*
der(14;21) (patient 13)	2164	0.55	0.32	0.32	0.32	0.36
Controls/mean (Vegetti <i>et al.</i> , 2000)	4243	0.13	0.06	0.14	0.13	0.03

**P* < 0.001.

of cells (Han *et al.*, 1992; Holmes and Martin, 1993; Rousseaux *et al.*, 1995). The proportion of chromosomally normal (balanced) and abnormal (unbalanced) sperm can be determined relatively accurately. Therefore, comparison of the meiotic behaviour of different Rob translocations becomes possible; and furthermore, when the same chromosome is involved in different Rob translocations, corresponding modes of segregation can easily be compared. In contrast to the meiotic behaviour of reciprocal translocations, which can vary according to the chromosomes and breakpoints involved (Rousseaux *et al.*, 1995; Guttenbach *et al.*, 1997; Van Hummelen *et al.*, 1997; Blanco *et al.*, 1998; Martini *et al.*, 1998; Estop *et al.*, 1999; Honda *et al.*, 1999; Van Assche *et al.*, 1999; Oliver-Bonet *et al.*, 2001; Geneix *et al.*, 2002), a similar segregation pattern could be expected in all Rob translocations because of its similar structural rearrangement.

Our data show that, in general, most of the spermatozoa in Rob translocation carriers are balanced for the chromosomes involved in the respective translocations and range from 76 to 89.47%.

In the group of the seven der(13;14) carriers, the rates of normal/balanced spermatozoa range from 78.40 to 89.47%, whereas the range of unbalanced gametes, resulting from the adjacent mode of segregation,

varies between 10.24 and 21.20%. In seven previous reports (Pellestor *et al.*, 1987; Martin, 1988; Escudero *et al.*, 2000; Honda *et al.*, 2000; Ogawa *et al.*, 2000; Morel *et al.*, 2001; Anton *et al.*, 2004), a total of 24 890 spermatozoa from 18 der(13;14) translocation carriers was analysed. The first two studies used the human–hamster inter-specific fertilization system, whereas Ogawa *et al.* (2000) used microinjected mouse oocytes with human sperm. The FISH technique was used in the other studies. Table VII summarizes the results of the different studies including our own. Our data confirm the results of earlier studies reporting a mean frequency of 85.25% (range: 73.50–91.10%) of gametes, resulting from an alternate segregation and a mean frequency of 13.90% (range: 7.70–26.5%) of spermatozoa, deriving from adjacent segregations. The wider range reported in other studies and compared with our own may be related to technical aspects, such as the number of cells analysed (human–hamster fertilization system versus FISH), the characteristics and the combination of the probes used (locus-specific, sub-telomeric or chromosome paints) and/or the specific scoring criteria used. There could have been patient-to-patient differences as well. Table VIII summarizes our data and the data of other reports on the Rob translocations der(13;15), der(14;21) and

Table VII. Review of literature of meiotic segregation of chromosomes in carriers of the Robertsonian (Rob) translocation der(13;14) (the values of alternate and adjacent types of segregation are expressed in %)

Reference	Number of cells	Segregation pattern	
		Alternate (%)	Adjacent (%)
Pellestor <i>et al.</i> (1987)*	78	92.30	7.70
Martin (1988)*	117	73.50	26.50
Ogawa <i>et al.</i> (2000)†	45	91.10	8.90
Escudero <i>et al.</i> (2000)‡			
Patient 1	1016	73.60	23.30
Patient 2	1006	77.40	19.10
Frydman <i>et al.</i> (2001)‡			
Patient 1	1045	91.00	9.00
Patient 2	1023	90.00	10.00
Patient 3	1008	87.10	12.90
Morel <i>et al.</i> (2001)‡			
Patient 1	2984	81.34	18.06
Patient 2	1109	82.60	16.32
Patient 3	1009	88.90	10.80
Anton <i>et al.</i> (2004)‡			
Patient 1	1361	86.48	12.56
Patient 2	2901	87.49	12.17
Patient 3	1012	83.00	14.53
Patient 4	1002	84.53	14.17
Patient 5	1272	88.13	11.40
Patient 6	6128	88.23	11.11
Patient 7	774	87.73	11.63
Total	Mean 1383	Mean 85.25	Mean 13.90
Present study‡			
Patient 1	500	81.81	18.60
Patient 2	500	78.40	21.20
Patient 3	1032	85.47	14.33
Patient 4	507	85.80	13.67
Patient 5	1001	83.52	16.48
Patient 6	1016	89.47	10.24
Patient 7	657	84.65	15.07

*Studies by the hamster egg–human sperm fusion technique heterospecific fertilization.

†Study by sperm injection into mouse oocytes.

‡Studies by fluorescence in-situ hybridization (FISH).

der(21;22). Our results are in agreement with the published data, and the same remarks as made above could explain the wider range of frequencies observed by the different authors.

We can conclude that our data and those already published support the existence of a similar meiotic behaviour of the rearranged chromosomes, independent of the chromosomes involved in the Rob translocation.

For the chromosomes involved in the Rob translocation, theoretically, the adjacent modes of segregation should yield the same rates. This was confirmed in this study since within groups, in each case, no statistical difference was observed between the sum of nullisomies and disomies between the two chromosomes studied (13 versus 14, 13 versus 15, etc.). However, it was remarkable that for most of the patients, a higher proportion of nullisomies than disomies was observed for chromosomes 13, 14, 15 and 22. In contrast, in the three carriers involving chromosome 21 in the Rob translocation, the disomy rates were always higher than the nullisomy (for patient 12: 40 versus 20; for patient 13: 40 versus 36 and for patient 14: 36 versus 33) (Table III).

Similar high rates of nullisomies 13 and 14 were reported by Escudero *et al.* (2000) and Morel *et al.* (2001). As suggested by Rousseaux *et al.* (1995), the observed high rates of nullisomies could be because of an artefactual overestimation, resulting from unequal hybridization efficiencies of both probes. However, because the same phenomenon was observed in the reports using the hamster ovum penetration test, it could also be suggested that survival of monosomic

Table VIII. Review of literature of meiotic segregation of chromosomes in carriers of the Robertsonian (Rob) translocations der(13;15), der(14;21) and der(21;22) (the values of alternate and adjacent types of segregation are expressed in %)

Type of Rob	Reference	Number of cells	Segregation pattern	
			Alternate	Adjacent
der(13;15)	Pellestor (1990)*	76	89.60	10.40
	Present study†			
	Patient 8	1109	82.70	17.00
der(14;21)	Patient 9	1021	76.00	23.40
	Balkan and Martin (1983)*	24	87.50	12.50
	Rousseaux <i>et al.</i> (1995)†	1116	72.20	12.00
	Honda <i>et al.</i> (2000)†	16 578	88.42	11.25
	Frydman <i>et al.</i> (2001)†			
Patient 1	1000	91.30	8.70	
Patient 2	1000	92.80	7.20	
Patient 3	1000	93.00	7.00	
Present study†				
Patient 12	996	87.90	11.80	
Patient 13	1102	85.70	12.60	
der(21;22)	Syme and Martin (1992)*	149	96.60	3.40
	Mennicke <i>et al.</i> (1997)†	350	60.00	40.00
	Present study†	1016	85.60	13.30

*Studies by the hamster egg–human sperm fusion technique heterospecific fertilization.

†Studies by fluorescence in-situ hybridization (FISH).

sperm versus disomic sperm is preferential. More studies, using three-colour FISH (=two probes for the chromosomes involved in the translocation and the third probe for another chromosome), are needed to confirm or infirm these observations.

Nullisomic or disomic gametes, resulting from the adjacent mode of segregation for the der(13;14) Rob translocation, are the underlying conditions for the formation of monosomic and trisomic embryos. If in der(13;14) carriers, the formation of nullisomic spermatozoa were a preferential mode of adjacent segregation, more monosomic 13 or 14 embryos would be observed. Recently, preimplantation genetic diagnosis (PGD) became possible for Rob carriers, and consequently the genetic constitution of embryos could be investigated. Escudero *et al.* (2000) investigated the meiotic segregation pattern of chromosomes 13 and 14 in 32 PGD embryos of two couples with a male Rob carrier. The authors did, in fact, find more embryos with monosomies than trisomies: three embryos with monosomy 13 (9.4%), two embryos with monosomy 14 (6.3%), one embryo with trisomy 14 (3.1%) and none with trisomy 13. This observation could also be confirmed at our Centre. Four PGD cycles for two couples with a male Rob carrier were performed in this study, and 26 embryos in total were investigated. Three monosomy 13 embryos (11.5%), three monosomy 14 embryos (11.5%), two trisomy 13 embryos (7.7%) and no trisomy 14 embryos were found. Despite the limited numbers of embryos, we could suggest that nullisomic sperm may be more likely to fertilize ova than disomic sperm or it could be that preferential survival of nullisomic sperm could be the basis of more monosomic embryos.

Independently of the chromosomes involved in the Rob translocation, all investigations agree that a vast majority (72.2–96.6%) of sperm originates by alternate segregation. This can be confirmed in all PGD cycles for carriers of Rob translocations (Conn *et al.*, 1998; Iwarsson *et al.*, 2000; Scriven *et al.*, 2001; Alves *et al.*, 2002).

The incidence of unbalanced constellations in prenatal diagnosis and in new-borns is much lower than that found in sperm and embryos (Boué and Gallano, 1984; Daniel *et al.*, 1986). In a European collaborative study, none of 230 mid-trimester prenatal diagnoses performed on pregnancies of der(13;14) translocation carriers had an unbalanced

karyotype (Boué and Gallano, 1984). For male carriers of der(14;21) translocations, a risk of 4.3% of imbalance at amniocentesis has been reported (Daniel *et al.*, 1989). Again, this frequency is much lower than the frequencies of unbalanced sperm in the above-mentioned studies including our own. A selection against unbalanced fetuses before mid-pregnancy explains these findings.

For every type of Rob translocation, 2:1 is the preferential mode of segregation. The 3:0 mode of segregation resulting in double trisomic or monosomic embryos is an extremely rare event. Diploid gametes resulting in triploid embryos are also infrequent (Egozcue *et al.*, 2002). Most probably, embryos carrying such cytogenetic aberrations are lost during early pregnancy.

Morel *et al.* (2001) suggested that the use of a third probe, specific for a chromosome not involved in the Rob translocation, could be used to distinguish 3:0 mode of segregation from diploid gametes. By using two-colour FISH, four signals from two probes are visible in both situations. Because two-colour FISH was used in all PGD cycles for Rob carriers, no discrimination between embryos derived from 3:0 segregation or diploidy could be made, and so far no information is available from early embryonic life. In this study, three translocation carriers were tested by using three-colour FISH (patients 7, 8 and 13; Table II). Besides the probes involved in the Rob translocation, a centromeric probe for chromosome 10 (labelled with spectrum aqua) was added in three different probe mixtures. For each patient, the frequency for diploid gametes was higher than gametes resulting from 3:0 segregation. The only gamete we observed with 3:0 mode of segregation was from the der(13;14) carrier, suggesting that this mode of segregation is indeed a rather infrequent event.

It is still not clear whether there is an inter-chromosomal effect of the rearranged chromosomes on the chromosomes not involved in the translocation present in Rob translocation carriers. Few reports mention the risk of such an effect. (Mercier *et al.*, 1998; Estop *et al.*, 2000; Vegetti *et al.*, 2000; Pellestor *et al.*, 2001). In this study, 14 050 spermatozoa were screened in five Rob carriers for two chromosome sets: 13 and 21 on the one hand and X, Y and 18 on the other. In our series, a significantly higher frequency for nullisomy of the sex chromosomes was observed for the der(13;14) carrier; both carriers of der(14;15) seemed to have a significantly higher frequency for disomy X–Y and one carrier of der(14;15), a significantly higher frequency for 18, X–Y diploidy. In three of five patients, significantly different frequencies observed support the existence of an inter-chromosomal effect on the sex chromosomes. By performing classical meiotic studies and synaptonemal complex studies, Luciani *et al.* (1984) and Navarro *et al.* (1991) found a non-random association at prophase I between the trivalent, at the short-arm regions of the non-fused chromosomes, and the sex chromosomes, in several Rob translocation males analysed. As suggested by Anton *et al.* (2004), inter-individual variations concerning the presence or absence of an inter-chromosomal effect that are observed among the carriers can be related to specific characteristics of the translocated chromosomes, e.g. satellite polymorphisms common in acrocentric chromosomes.

To the best of our knowledge, the present contribution reports the largest series of data concerning modes of segregation determined in sperm of different Rob translocation carriers. The results of our study, as well as those of other studies, suggest that in all Rob translocation carriers, the meiotic segregation behaviour is similar and occurs non-randomly. Carriers with normal sperm parameters and OAT carriers do not feature different frequencies of imbalances, suggesting that segregation patterns and impairment of spermatogenesis are most probably independent processes. Most of the spermatozoa originate through alternate segregation. Analysis of meiotic prophase cells in heterozygous carriers of different Rob translocations showed that the

predominance of a preferential *cis*-configuration of the meiotic trivalent structure could promote alternate segregation (Vidal *et al.*, 1982; Luciani *et al.*, 1984; Templado *et al.*, 1984; Rosenmann *et al.*, 1985).

In spite of high numbers of normal/balanced frequencies (mean: 84.22%; Tables III, IV, VII and VIII), there is still to be much unbalanced spermatozoa resulting from adjacent mode of segregation (mean: 15.08%; Tables III, IV, VII and VIII). As previously suggested by Frydman *et al.* (2001), these relatively high frequencies of unbalanced spermatozoa do justify PGD for the translocation, in combination with aneuploidy screening, for chromosomes involved in non-lethal trisomies for these couples, especially in infertile translocation carriers. Through selective and preferentially single-embryo transfer, these couples can, if pregnant, avoid early spontaneous loss of unbalanced fetuses as well as the termination of an unbalanced pregnancy. Moreover, their chance of becoming pregnant may increase.

Finally, we can conclude that, despite slight inter-individual differences, carriers of Rob translocations have a non-random homogeneous segregation pattern with a clearly preferentially alternate segregation leading to a mean of 84.24% (range: 72.20–96.60%) of balanced gametes. These data are useful for genetic counselling purposes in the case of male Rob translocation carriers. We further believe that an inter-chromosomal effect in Rob translocations should not be underestimated and further studies should be performed to understand this phenomenon.

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