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ARTICLE

Cytogenetic analysis of sperm chromosomes and sperm nuclei in a male heterozygous for a reciprocal translocation t(5;7)(q21;q32) by *in situ* hybridisation

P Cifuentes[1], J Navarro[1], J Blanco[2], F Vidal[2], L Míguez[3], J Egozcue[2] and J Benet[1]

[1]*Unitat de Biologia, Facultat de Medicina*
[2]*Unitat de Biologia Cellular, Facultat de Ciències, Departament de Biologia Cellular i Fisiologia, Universitat Autònoma de Barcelona*
[3]*Diagnòstic Prenatal, Anexe Clinica Quirón, Barcelona, Spain*

We have studied the meiotic segregation of a reciprocal translocation t(5;7)(q21;q32) in a male carrier, using the human sperm–hamster oocyte fusion technique and the whole chromosome painting. A total of 296 sperm complements were analysed by dual chromosome painting. The frequencies of alternate, adjacent-1, adjacent-2 and 3:1 segregation were 49.7%, 32.4%, 16.2% and 1.7% respectively. Aneuploidy frequencies for chromosomes not involved in the translocation were determined by FISH on decondensed sperm heads using probes from chromosomes X, Y, 6, 18 and 21. A total of 20 118 spermatozoa was analysed, 10 201 by two-colour FISH (probes for chromosomes 6 and 21) and 9917 by three-colour FISH (probes for chromosomes X, Y, and 18). There was no evidence of an interchromosomal effect, since disomy frequencies were within the range of normal controls.

Keywords: sperm chromosome; meiotic segregation; chromosome rearrangements; *in situ* hybridisation; whole chromosome painting; offspring risk

Introduction

Reciprocal translocations are the most frequent structural abnormalities reported in newborns (0.092%).[1] Balanced carriers of a reciprocal translocation have an increased risk of producing unbalanced offspring and spontaneous abortions. Direct analysis of the chromosome constitution of human spermatozoa after penetration of hamster oocytes,[2] allows us to determine the predominant segregation types and to establish better the risk for the offspring. To date 39 carriers of reciprocal translocations have been studied using this system and analysed by classical cytogenetic techniques, such as Q-banding, G-banding or R-banding (reviewed by Martin,[3] Estop *et al*,[4] Martin and Spriggs[5]). The analysis of human sperm chromosomes has shown that the percentage of unbalanced gametes may vary between 19% and 87%, indicating that the frequencies of the different types of unbalanced gametes depend on the chromosomes involved, the breakpoints, and the number and location of chiasmata.

In recent years, some segregation studies of reciprocal translocation carriers have been accomplished by the use of fluorescent *in situ* hybridisation (FISH) in

Correspondence: Piedad Cifuentes Moraleda, Unitat de Biologia, Facultat de Medicina, Departament de Biologia Cellular I Fisiologia, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Spain. Tel: 34 935 81 11 75; Fax: 34 935 81 10 25; E-mail: biolmed@cc.uab.es
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decondensed sperm nuclei.[6,7] However, with this approach it is not possible to distinguish individually all segregation products. The reason is that even when locus-specific or painting probes are used, some segregation products produce identical signals. The use of a higher number of probes improves the results,[8,9] but still some technical difficulties may be encountered.[9] On the other hand, the relatively fast analysis of a higher number of cells makes this technique ideal for the study of aneuploidy and possible interchromosomal effect.[10]

In this report we describe the results of a sperm segregation analysis in sperm chromosomes using a whole chromosome painting technique and the results of the study of aneuploidy by FISH in decondensed sperm in a carrier of a reciprocal translocation t(5;7)(q21;q32).

Materials and Methods

A man with a 46, XY, –5, –7, + t(5;7)(q21;q32) karyotype was ascertained after his wife underwent amniocentesis for advanced maternal age. The prenatal diagnostic indicate that the child carried the same balanced translocation as his father. There was a history of miscarriage in the father's family. A semen sample was obtained from this man, aliquoted and cryopreserved in liquid nitrogen. Consent was obtained from all family members studied, and the protocol of the study has been approved by our institutional ethics committee.

Preparation of Sperm Chromosomes

Sperm chromosomes were obtained after culture of zona-free hamster oocytes penetrated by human spermatozoa according to a procedure described elsewhere[11] with some modifications:[12]

1) we used thawed samples;
2) capacitation of spermatozoa was increased by treatment with ionophore A23187;
3) we used vinblastine as an antimitotic instead of colcemid.

Slides were cryopreserved at –20°C until hybridisation.

Preparation of Sperm Nuclei

The semen sample to be used was thawed and washed with 0.9% NaCl to eliminate the cryoprotectant. The sample was fixed and decondensed following the protocol described by Vidal *et al*.[13] Slides were preserved at –20°C until hybridisation.

Probes and Hybridisation Procedure

Whole chromosome painting was performed using chromosome-specific probes for chromosomes 5 and 7 (VysisInc, Downers Grove, IL, USA; Biovation Ltd, Aberdeen, UK). Slides with human sperm chromosome spreads were refixed with 1% paraformaldehyde in PBS/50 mM $MgCl_2$ for 10 min and dehydrated in an ethanol series (70%, 95% and 100%). The probe mixture was dropped on to each slide, a coverslip was added and sealed with rubber cement. Combined denaturation was performed at 75°C for 5 min. After incubation, post-hybridisation washes were performed according to manufacturer's instructions. Slides were dehydrated in an ethanol series (70%, 95% and 100%) and counterstained and mounted with antifade solution (Vector Lab Inc, Burlingame, CA, USA) containing DAPI (Sigma, Madrid, Spain).

Two and three-colour FISH were performed in decondensed sperm nuclei, using centromere and locus specific probes. Two-colour FISH was performed to determine the frequency of disomy for autosomes, with a centromeric probe for chromosome 6, directly labelled with Spectrum Green (CEP 6, Vysis Inc.) used as a ploidy and hybridisation control, and a locus-specific probe for chromosome 21, directly labelled with Spectrum Orange (LSI 21, Vysis Inc.). To determine the frequency of disomy for the sex chromosomes, three-colour FISH was performed using centromere probes directly labelled for chromosome X (CEP X Spectrum Green, Vysis Inc.), chromosome Y (CEP Y Spectrum Orange, Vysis Inc.) and chromosome 18, (CEP 18 Spectrum Aqua, Vysis Inc.) used as a ploidy and hybridisation control. The hybridisation protocol followed manufacturer's instructions.

Scoring and Digital Imaging Storage

Slides were observed with an Olympus AX70 photomicroscope (Olympus Optical Co., Hamburg, Germany) equipped with epifluorescence optics. The Olympus filter set included three simple filters for visualising DAPI, FITC and Cy3 fluorescence, and a fourth triple filter for visualising DAPI/FITC/PI. The images were analysed with a Cytovision system (Applied Imaging, Sunderland, UK).

For sperm head analysis, only nuclei unequivocally identified as decondensed sperm because of their oval morphology and/or presence of a tail were scored. To decrease the subjectivity of the observations, the criteria described by[14] were used.

Data were statistically analysed by an InStat 2.01 program (Graph Pad, San Diego, CA, USA) using a χ^2 test.

Results

Segregation analysis was performed on a total of 296 sperm complements by dual chromosome painting. All possible 2:2 and some 3:1 segregations were observed (Figure 1 a–e). Of the 296 sperm complements, 147 (49.7%) resulted from alternate segregation, of which 83 (28.0%) contained normal chromosomes and 64 (21.6%) the balanced translocation; 96 (32.4%) complements resulted from adjacent-1 segregation, 48 (16.2%) from adjacent-2 segregation and 5 (1.7%) from 3:1 segregation (Table 1). In the products of alternate segregation, the frequency of complements containing normal chromosomes was not significantly different from the frequency of complements with a balanced translocation (χ^2 test, P = 0.3843). The theoretical risk

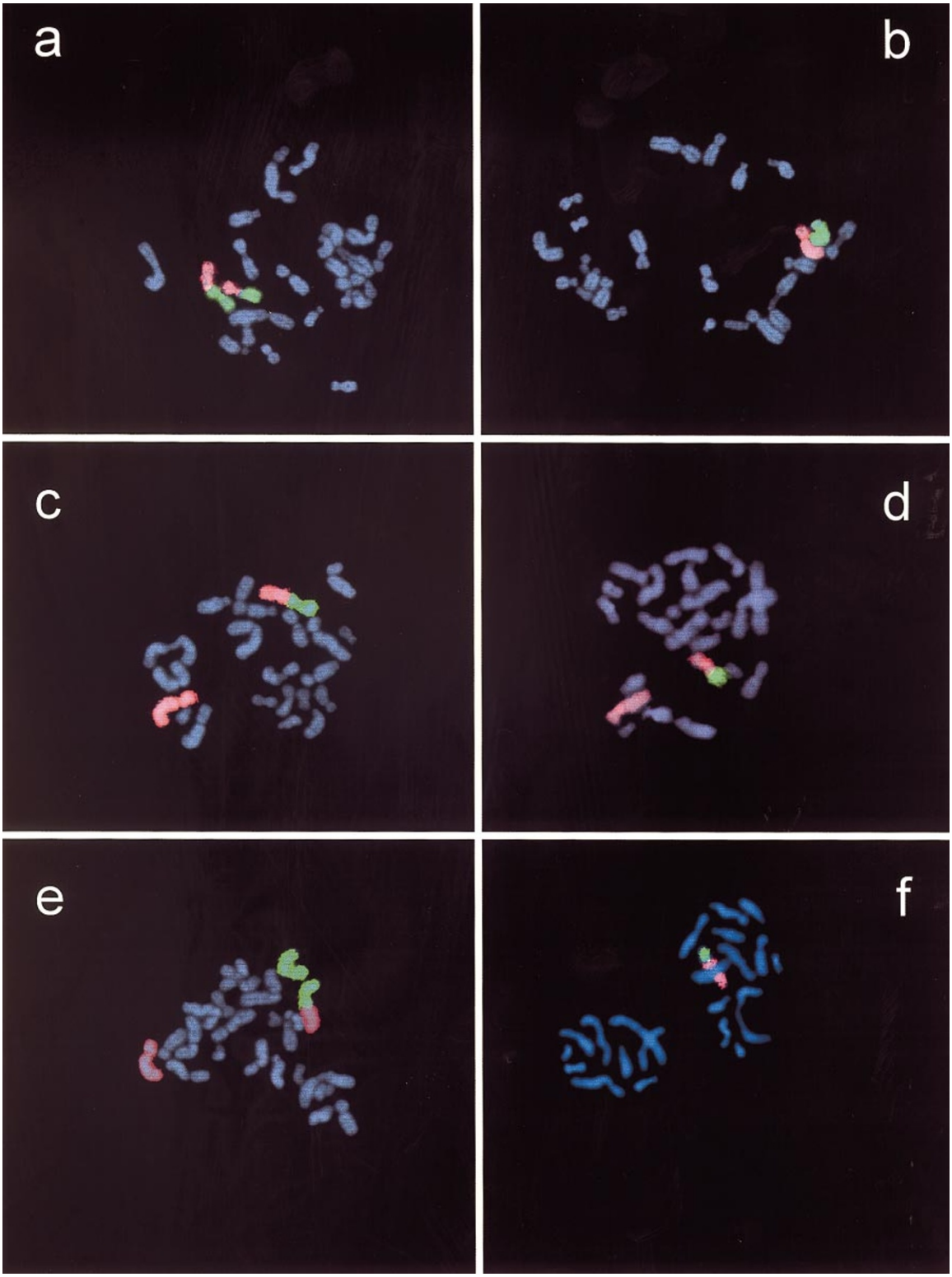

Figure 1 *Sperm chromosome complements containing different segregation products. Whole chromosome painting of chromosome 5 (red) and 7 (green).* **a)** *Balanced.* **b)** *Normal.* **c)** *Adjacent 1.* **d)** *Adjacent 2.* **e)** *3:1 containing two normal chromosomes 5 and 7 and a translocated chromosome der(7); this metaphase contains only 23 chromosomes because chromosome 19 is missing.* **f)** *3:1 containing a single chromosome der(5).*

of unbalanced offspring produced by this carrier is 50.3% (Table 1).

Aneuploidy frequencies of chromosomes not involved in the translocation were determinated by FISH on decondensed sperm nuclei. Centromere probes from chromosomes X, Y, 6, 18, and a locus specific probe for chromosome 21 were used. A total of 20 118 spermatozoa, from the t(5;7) carrier, was analysed, 10 201 by two-colour FISH and 9917 by three-colour FISH. A total of 28 044 spermatozoa, from nine control donors, analysed by two-colour FISH, and 50 572 spermatozoa, from five control donors, analysed by three-colour FISH[14,15] were used as controls for this study. The frequencies of gonosomal and autosomal hyperploidy detected in the translocation carrier and in the controls are shown in Table 2. There were no statistically significant differences in the frequencies of autosomal disomy between the controls and the translocation carrier. For sex chromosome disomies, significantly lower frequencies were observed in the translocation carrier than in controls (Table 2). The total frequency of diploid sperm in the translocation carrier (0.1%) was also lower than in controls (0.26%) (Table 2). The sex ratio was determined in 9917 cells and was not different from the expected 1:1 (χ^2 test, P = 0.8153).

Table 1 Meiotic segregation of sperm chromosomes in a t(5;7)

Segregation	*Type*	*Number*	*Percent*
Alternate	normal	83	28.0%
	balanced	64	21.6%
Subtotal: balanced		147	49.7%
Adjacent 1	5;der(7)	47	15.9%
	7;der(5)	49	16.6%
Adjacent 2	5;der(5)	29	9.8%
	7;der(7)	19	6.4%
3:1		5	1.7%
Subtotal: unbalanced		149	50.3%
Total		296	100%

Discussion

The production of normal, balanced or unbalanced gametes in carriers of reciprocal translocations depends on the location of the breakpoints and on the characteristics of the chromosomes involved (length, presence or absence of heterochromatin, chiasma formation).[16–19] In most cases, such as the one studied by us, there is a predominance of 2:2 segregations, corresponding mainly to the alternate and adjacent 1 types as observed in those translocations that produce ring configurations.[20] The incidence of 3:1 segregations[20] is directly related to the formation of open configurations, such as chains; 4:0 segregations have seldom been described in sperm chromosome studies,[3,17,21] and their products have never been found in newborns. FISH studies in sperm have been unable, so far, to differentiate between 4:0 segregations and diploid sperm because the number of probes used was limited to the chromosomes involved in the translocation.

So far, including the present work, 40 reciprocal translocations have been studied using the human–hamster system, confirming the tendencies indicated

Table 2 Frequency of hyperploid and diploid sperm

Chromosome constitution	*t(5;7) %*	*Controls %(±SD)*	χ^2
Normal:			
23 X	50.96	49.98(±0.91)[b]	
23 Y	47.28	48.70(±1.18)[b]	
Gonosomal hyperploidy:			
X-X-18	0.01	0.10(±0.04)[b]	
Y-Y-18	0.07	0.16(±0.06)[b]	
X-Y-18	0.07	0.11(±0.05)[b]	
Total	0.15	0.37	0.0039
Autosomal hyperploidy:			
6-6-21	0.09	0.13(±0.11)[a]	0.8159
18-18-X or 18-18-Y	0.03	0.10(±0.03)[b]	0.0614
6-21-21	0.23	0.37(±0.12)[a]	0.1937
Diploidy:			
X-X-18-18 or Y-Y-18-18 or X-Y-18-18	0.09	0.25(±0.15)[b]	0.0028
6-6-21-21	0.11	0.27(±0.10)[a]	0.0039

[a]Ref. 14; [b]Ref. 15

above. Most segregations have been of the 2:2 type with mean frequencies of 45.6% for alternate and 37.1% for adjacent 1 segregation.[5] Furthermore, epidemiological studies[18] indicate that most cases of unbalanced offspring result from adjacent 1 segregations. This is not unexpected[19,22] because the adjacent 2 and 3:1 types combine abnormal segregation and non-disjunction and, other than to the configuration of the tetravalent, may also be related to problems in the resolution of chiasmata.[8] The mean risk of producing unbalanced offspring has been established at 54.4%[22] which is comparable to the figure obtained by us in this case (50.3%).

However, these frequencies are much higher than the ones observed at birth[23] and underline the problems encountered when evaluating the risk at birth *vs* evaluating it at conception. Each estimate is valid on its own, and they should not be mixed, much less confused.

One further translocation affecting chromosomes 5 and 7 has been described in the literature; it was a t(5;7)(q13;p15.1),[3] and the results of segregation were different from those observed in our case: 40.2% alternate, 26.2% adjacent 1, 16.6% adjacent 2 and 17.0% 3:1 segregation. However, the breakpoints in these two translocations were different and, based on the distribution of hot regions for chiasma formation,[24,25] these differences could be related to the probable formation of interstitial chiasmata in our case and to the probable absence of interstitial chiasmata in the other (Figure 2).

As for the possible existence of preferential segregation products in spermatozoa, recently Van Hummelen *et al*,[8] in a study of decondensed sperm heads using multicolour FISH, detected a higher number of spermatozoa containing short translocated segments after adjacent 1 segregation or its normal equivalent after adjacent 2 segregation. This distortion was explained by the authors[8] as the non-resolution of chiasmata at meiosis I that would be directly proportional to the length of the translocated segment. However, a study of the segregation products in sperm chromosomes of translocation carriers (Table 3) does not support a distortion towards sperm carriers of short translocated segments (assuming the proportion between products = 1, $\chi^2 = 10.531$ for 18 freedom degree, $P = 0.9132$). Furthermore, a recent study[9] on decondensed sperm heads in the carrier of a t(3;11) shows the opposite, ie a distortion towards products containing the longer translocated segment. Thus, more cases will have to be studied to determine whether there is a general tendency towards the inclusion of one or other type of translocation product in viable sperm, the distribution is random, or it varies with each individual case.

For some time it has been suggested that interchromosomal effects[26] could increase the rate of other chromosome abnormalities, such as regular non-disjunction in carriers of structural chromosome reorganisations. However, among the 40 reciprocal translocations analysed so far using the human–hamster system, this interchromosomal effect has only been observed in the carrier of a double translocation.[17] Although only 23 metaphases were studied, this patient had a 70% increase in the frequency of chromosome abnormalities (numerical) not directly related to the translocations.

Interchromosomal effects are difficult to evaluate, because one has to study at least 150–400 sperm metaphases to observe a three to two-fold increase in disomies,[19] and so far most published cases are based on the analysis of fewer than 150 metaphases. Therefore to determine the possible existence of an interchromosomal effect in our patient, we analysed decondensed sperm heads by FISH using autosomal (6, 18, 21) and gonosomal (X, Y) fluorescent probes, combining 6 and 21 on the one hand and 18, X and Y on the other. In this way it is possible to analyse thousands of sperm heads. Although the chromosomes analysed (21, X and Y) are those most frequently involved in viable non-disjunction events[27] we did not find any increase in the frequency of disomy for any of the chromosomes analysed (21, X, Y) or for the controls used (6, 18). We did not find any significant increase in the level of diploidy either, although it seems to be the most common anomaly when meiosis is disrupted.[28] Interchromosomal effects such as those producing meiotic arrest or meiotic disruption resulting in diploidy could be related to more asymmetrical reorganisations or to other meiotic problems such as synaptic anomalies, resulting in higher degrees of unbalance, be they viable (diploidy) or unviable (apoptosis and meiotic arrest).

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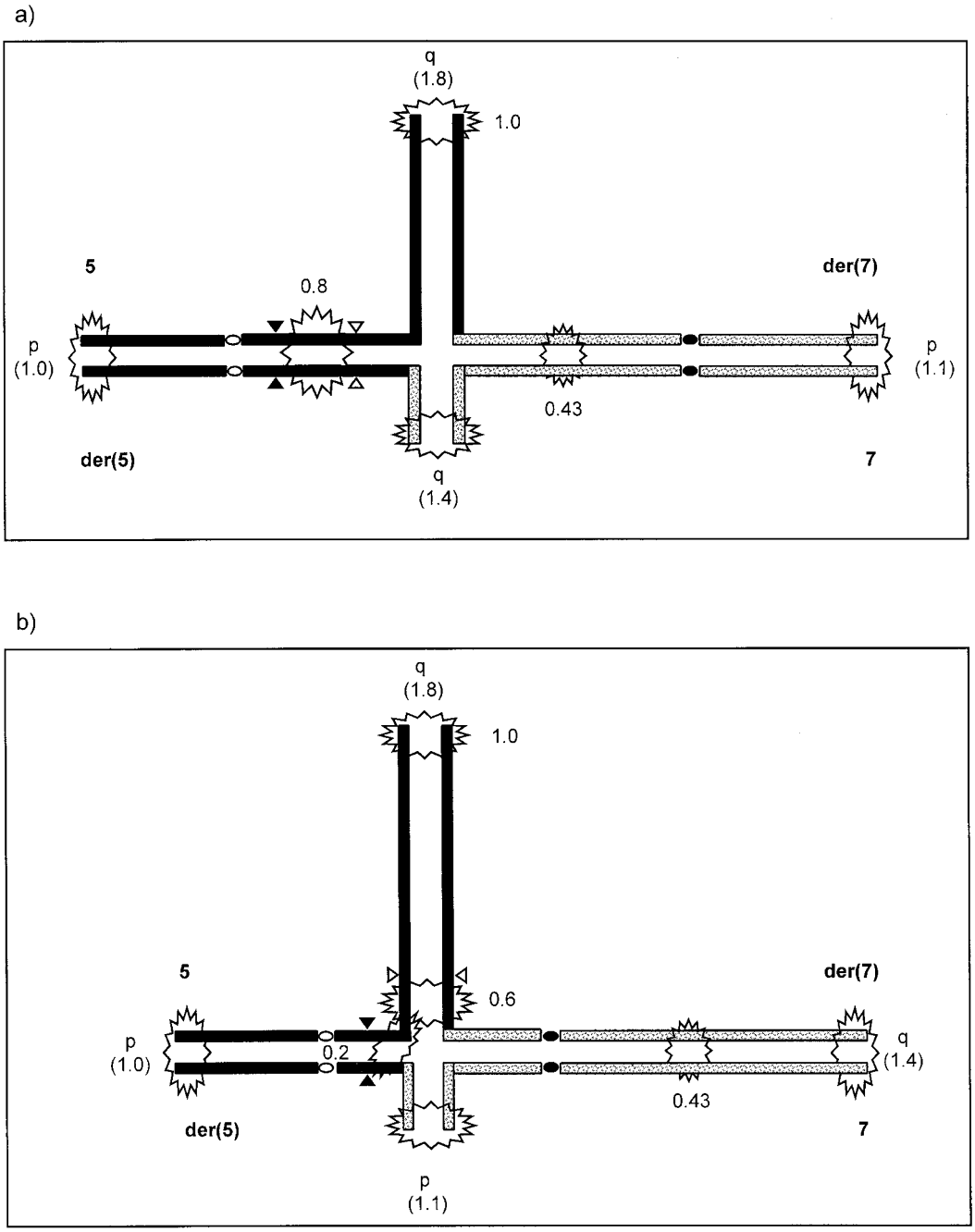

Figure 2 *Tetravalent figure at meiosis I* **a)** *for t(5;7)(q21;q32) and* **b)** *for t(5;7)(q13;p15.1). High recombination regions and mean chiasma frequency for each arm according to Laurie and Hultén*[24,25] *are indicated.*

Table 3 Ratio distortion of reciprocal sperm products from adjacent 1 segregation in different translocation carriers

Translocation carrier	*No. of sperm analysed*	*Short/long translocated genotype*	*Ratio distortion*	*References*
Hamster technique:				
t(1;2)(q32;q36)	105	–1, +der(1)/–2, +der(2)	1.2	20
t(1;4)(p36.2;q31.3)	90	–4, +der(4)/–1, +der(1)	0.73	25
t(1;4)(p36.2;q31.3)	115	–4, +der(4)/–1, +der(1)	0.57	25
t(1;9)(q22;q31)	173	–1, +der(1)/–9, +der(9)	1.24	29
t(1;11)(p36.3;q13.1)	548	–11, +der(11)/–1, +der(1)	0.97	30
t(2;9)(q21;p22)	208	–2, +der(2)/–9, +der(9)	0.64	31
t(2;18)(p21;q11.2)	165	–18, +der(18)/–2, +der(2)	0.81	3
t(3;11)(q25.3;q25)	262	–3, +der(3)/–11, +der(11)	0.96	32
t(3;16)(p23;q24)	201	–3, +der(3)/–16, +der(16)	1.07	33
t(4;6)(q28;p23)	158	–4, +der(4)/–6, +der(6)	1	31
t(5;7)(q13;p15.1)	157	–5, +der(5)/–7, +der(7)	0.78	3
t(5;7)(q21;q32)	296	–5, +der(5)/–7, +der(7)	0.96	this study
t(7;20)(q33.2;p13)	263	–7, +der(7)/–20, +der(20)	0.72	32
t(8;15)(p22;q21)	226	–15, +der(15)/–8, +der(8)	0.59	33
t(9;10)(q34;q11)	168	–10, +der(10)/–9, +der(9)	1.7	34
t(11;17)(p11.2;q21.3)	184	–11, +der(11)/–17, +der(17)	1.1	30
t(12;20)(q24.3;q11)	113	–20, +der(20)/–12, +der(12)	1.24	35
t(15;22)(q26.1;q11.2)	147	–22, +der(22)/–15, +der(15)	1.07	32
t(16;19)(q11.1;q13.3)	172	–6, +der(16)/–19, +der(19)	1	29
Decondensed sperm:				
t(1;10)(p22;q22)	4036	–1, +der(1)/–10, +der(10)	1.28	8
t(3;11)(q27.3;q24.3)	4029	–1, +der(1)/–2, +der(2)	0.77	9

Only studies that analysed ≥90 sperm complements are included

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