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Meiotic Segregation, Recombination, and Gamete Aneuploidy Assessed in a t(1;10)(p22.1;q22.3) Reciprocal Translocation Carrier by Three- and Four-Probe Multicolor FISH in Sperm

Paul Van Hummelen, David Manchester, Xiu Lowe, and Andrew J. Wyrobek

Lawrence Livermore National Laboratory, Livermore, California

Summary

Meiotic segregation, recombination, and aneuploidy was assessed for sperm from a t(1;10)(p22.1;q22.3) reciprocal translocation carrier, by use of two multicolor FISH methods. The first method utilized three DNA probes (a telomeric and a centromeric probe on chromosome 1 plus a centromeric probe on chromosome 10) to analyze segregation patterns, in sperm, of the chromosomes involved in the translocation. The aggregate frequency of sperm products from alternate and adjacent I segregation was 90.5%, and the total frequency of normal and chromosomally balanced sperm was 48.1%. The frequencies of sperm products from adjacent II segregation and from 3:1 segregation were 4.9% and 3.9%, respectively. Reciprocal sperm products from adjacent I segregation deviated significantly from the expected 1:1 ratio ($P < .0001$). Our assay allowed us to evaluate recombination events in the interstitial segments at adjacent II segregation. The frequencies of sperm products resulting from interstitial recombination in chromosome 10 were significantly higher than those resulting from interstitial recombination in chromosome 1 ($P < .006$). No evidence of an interchromosomal effect on aneuploidy was found by use of a second FISH method that simultaneously utilized four chromosome-specific DNA probes to quantify the frequencies of aneuploid sperm for chromosomes X, Y, 18, and 21. However, a significant higher frequency of diploid sperm was detected in the translocation carrier than was detected in chromosomally normal and healthy controls. This study illustrates the advantages of multicolor FISH for assessment of the reproductive risk associated with translocation carriers and for investigation of the mechanisms of meiotic segregation of chromosomes.

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Address for correspondence and reprints: Dr. Paul Van Hummelen, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, L-452, 7000 East Avenue, Livermore, CA 94550. E-mail: vanhummelen1@poptart.LLNL.gov

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Introduction

Understanding the mechanisms of meiotic segregation of reciprocal translocations is of importance for estimation of the risk of pregnancy loss and of birth defects, for carriers. Reciprocal translocations form quadrivalents during meiosis I, which result in high frequencies of chromosomally imbalanced sperm (Hultén 1974; Burns et al. 1986). Proper segregation of the homologous centromeres of the quadrivalent chromosomes occurs after either alternate segregation or adjacent I segregation, whereas in adjacent II segregation the homologous centromeres migrate to the same poles. Partial or complete nondisjunction of the quadrivalent structure will lead to 3:1 segregation or to 4:0 segregation.

The proportions of balanced and unbalanced gametes of male carriers of reciprocal translocations have been estimated elsewhere from the analysis of quadrivalent structures at meiosis I metaphases, obtained from testicular biopsies (Goldman and Hultén 1993*a,* 1993*b*). Chromosome painting was found to enhance the identification of translocated chromosomes (Goldman and Hultén 1992). Large proportions of Meiosis I cells had at least one interstitial chiasma, which would result in dimorphic chromosomes bearing one normal and one translocated chromatid (Goldman and Hultén 1993*a*). Thus, alternate and adjacent I segregation types would generate the same sperm genotypes, depending on whether interstitial chiasmata were present. A drawback of the analyses of meiotic metaphase, however, is that the frequencies of various sperm products and the roles of selection can be inferred only.

Direct investigation of human-sperm chromosomes became possible by use of the human-sperm/hamster-oocyte cytogenetic technique (the hamster technique) (for review, see Estop et al. 1995; Martin and Spriggs 1995). By use of this method, it was shown that the majority of sperm products was predominantly normal or balanced and that the proportions of balanced and unbalanced sperm products in translocation carriers were consistent with the predictions from meiotic metaphase analysis. However, the hamster technique is a tedious and time consuming method that does not allow for the analysis of large numbers of cells.

For the current work, sperm from a t(1;10)-(p22.1;q22.3) reciprocal translocation carrier were analyzed by two multicolor FISH procedures. The first procedure was designed to assess all segregation classes detected by the hamster technique and utilized a telomeric and a centromeric probe on chromosome 1 plus a centromeric probe on chromosome 10. In contrast, the previous studies of sperm FISH, in translocation carriers, were limited to centromere detection and could not differentiate chromosomally balanced from imbalanced sperm resulting from alternate/adjacent I segregation (Spriggs and Martin 1994; Rousseaux et al. 1995).

It has been suggested, although never shown, by several groups (Aurias et al. 1978; Rousseaux et al. 1995) that the presence of reciprocal translocations may increase the aneuploidy frequencies of chromosomes not involved in the translocation (interchromosomal effect). Reciprocal translocations may influence the meiotic progression by altering meiotic recombination or chiasmata formation, which are crucial for proper segregation and which may serve as checkpoints for meiotic progression (Kleckner 1996). To investigate this hypothesis, we developed a new four-chromosome FISH assay, to determine whether the t(1;10) translocation carrier has polyploidy and interchromosomal effects on aneuploidy, for chromosomes X, Y, 18, or 21. These four chromosomes were chosen because they are unrelated to the translocation and represent the majority of the viable aneuploid offspring in humans (Hecht and Hecht 1987).

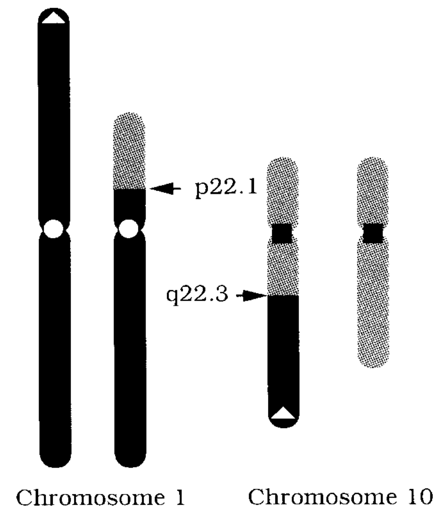

Figure 1 Illustration of t(1;10)(p22.1;q22.3) breakpoints and of the positions of the DNA probes used. An unblackened circle (○) indicates D1Z5 labeled in green; an unblackened triangle (△) indicates D1Z2 labeled in red; and a blackened square (■) indicates D10Z1 labeled in yellow.

Donors, Material, and Methods

Donors

Semen samples were provided by a t(1;10)-(p22.1;q22.3) translocation carrier and by two chromosomally normal donors. The carrier was 40 years of age and has two healthy daughters, one of whom also is a carrier. The control donors, A (43 y) and I (47 y), were part of an anonymous sperm-donor program (Lawrence Livermore National Laboratory) and reported no notable health problems or exposures to medical or environmental hazards. Donors A and I were evaluated previously for both structural and numerical chromosome abnormalities, by use of the hamster technique (Brandriff et al. 1988; Brandriff and Gordon 1990) and the sperm FISH aneuploidy assay (Robbins et al. 1993, 1995; Wyrobek et al. 1994; Van Hummelen et al. 1996). All donors gave their informed consent prior to inclusion in the study (Lawrence Livermore National Laboratory, Institutional Review Board, protocols 90-108 and 95-111; Colorado Multiple Institutional Review Board, protocol 95-234).

Pretreatment and Hybridization

Semen was aliquoted without any washing or centrifugation and was stored at −80°C until used. Semen samples were thawed at room temperature, and a volume of ~7 μl was smeared onto slides and air-dried overnight. The smears were pretreated by incubation for 30 min in 10 mM DTT (Chemical Abstracts Service [CAS] 27565-41-9) in a 0.1-M Tris-HCl solution (on ice), followed by 30 min in 4 mM lithium diiodosalicylate (CAS 653-14-5) in a 0.1-M Tris-HCl solution, at room temperature (Wyrobek et al. 1990; Robbins et al. 1993, 1995). The slides were allowed to air-dry. Hybridizations were performed as described in the study by Van Hummelen et al. (1996).

Probes and Analyses

Two separate sperm FISH analyses were performed (table 1; fig. 1). First, a three-probe segregation assay was used to detect the meiotic segregation products of the translocation carrier. Three DNA probes were utilized for this assay—two probes for chromosome 1 (D1Z5, a biotin-labeled alpha centromeric probe, and D1Z2, a p36.3 digoxigenin-labeled midisatellite probe) and a centromeric probe for chromosome 10 (D10Z1, a mixed biotin-labeled and digoxigenin-labeled probe). Second, a four-probe aneuploidy assay utilized a combination of DNA probes to estimate the aneuploidy frequencies of four chromosomes not involved in the translocations: an alpha satellite for chromosome X (CEPX, Spectrum Aqua™ labeled), an alpha satellite for chromosome Y (CyProbe Y, 50:50 mix of Cy 3 labeled and FluorX labeled), an alpha satellite for chromosome 18 (CyProbe 18, FluorX labeled), and a specific locus probe for chromosome 21 (LSI21, locus 21q22.13-q22.2 containing D21S259, D21S341, and D21S342; Spectrum Orange™ labeled).

Table 1

DNA Probes and FISH Labeling Strategy Used for Sperm Segregation and Aneuploidy Analyses

Analysis Type and Probe	Target DNA	Label	Color
Three-probe segregation assay:			
D1Z5[a]	Alpha centromere 1	Biotin (fluorescein isothiocyanate)	Green
D1Z2[a]	Midisatellite 1 (p36.3)	Digoxigenin (rhodamine)	Red
D10Z1[a]	Alpha centromere 10	Biotin (fluorescein isothiocyanate) and digoxigenin (rhodamine)	Yellow
Four-probe aneuploidy assay:			
CEPX[b]	Alpha centromere X	Spectrum Aqua	Blue
CyProbe Y[c]	Alpha centromere Y	Cy 3 and FluorX	Yellow
CyProbe 18[c]	Alpha centromere 18	FluorX	Green
LSI21[b]	Locus 21q22.13-q22.2	Spectrum Orange	Red

NOTE.—Nuclei were counterstained with DAPI.
[a] Oncor.
[b] Vysis.
[c] Amersham.

Scoring was performed on coded slides, by use of a Zeiss Axioplan fluorescence microscope with a 100 × Plan-NEOFLUAR Ph3-phase objective. Coding was done by a third party, who was not involved in the scoring. The scoring was conducted under strict criteria (Robbins et al. 1995; Van Hummelen et al. 1996): two fluorescence domains of the same color had to be separated by a distance of more than one-half the diameter of a fluorescence domain within that cell, in order to be recorded as two signals. For the D1Z5 green signal, this criterion was not followed strictly because the centromeric domain of chromosome 1 was sometimes less well defined, when compared with the other signals. Nuclei with abnormal fluorescent phenotypes were analyzed under phase contrast illumination (with the same objective) to verify the presence of the sperm tail. Cells with diploid fluorescence phenotypes were recorded as diploid sperm only when a sperm tail was present.

In the three-probe segregation, 4,036 cells (2,000 cells per slide) were scored on two separately hybridized and coded slides with samples from the translocation carrier. Abbreviations were used to denote the fluorescence phenotypes of the sperm: "A" (i.e., alpha) was used for the presence of the D1Z5 green signal, "M" (i.e., midi) for the presence of the D1Z2 red signal, "10" for the presence of the D10Z1 yellow signal, and "O" for the absence of an expected signal. For example, both normal and balanced sperm have one signal of each color, that is, a fluorescence phenotype of A-M-10. Other fluorescent phenotypes represent chromosomally imbalanced sperm; for example, A-M-M-10 represents a sperm with the genotype 23,−10,+der(10), which lacks the normal chromosome 10 (−10) but carries the translocated chromosome 10 (+der[10]).

In the four-probe aneuploidy assay, 10,000 cells per donor were scored per slide, by use of the following sequence: slides were coded; 5,000 cells were scored; slides were recoded; and then an additional 5,000 cells were scored on a different area of the same slide. For both FISH analyses, score data were entered into a database only if the two scores (from the same slide) were not statistically different, by use of 2 × 2 contingency tables (Snedecor and Cochran 1967). Otherwise, the slides were recoded and scored again. χ^2 analyses for one sample (Spence et al. 1983) were used to analyze the differences in frequencies among the categories of fluorescent phenotypes within a sample or among the same categories from different donors.

Results

Analysis of Sperm Segregation Products of a t(1;10) Translocation Carrier

Figure 2 illustrates the predicted sperm products after alternate, adjacent I, and adjacent II segregation at meiosis I in the t(1;10) translocation carrier, and table 2 lists the observed frequencies of the respective fluorescent sperm phenotypes. One sperm per ∼4,000, or ∼0.02%, failed to show any fluorescent domain. This frequency was not significantly higher than that observed in normal men, by use of the same assay (1 per 10^4) or by use of a similar assay published earlier (0.3 per 10^4; Van Hummelen et al. 1996). This apparently lower hybridization efficiency, however, did not significantly inflate the frequencies of the other categories.

The largest single group of sperm observed (48.1%) had the fluorescent phenotype A-M-10, which represents either normal or chromosomally balanced sperm generated by alternate segregation (table 2; fig. 2). However, this fluorescence phenotype also was produced by

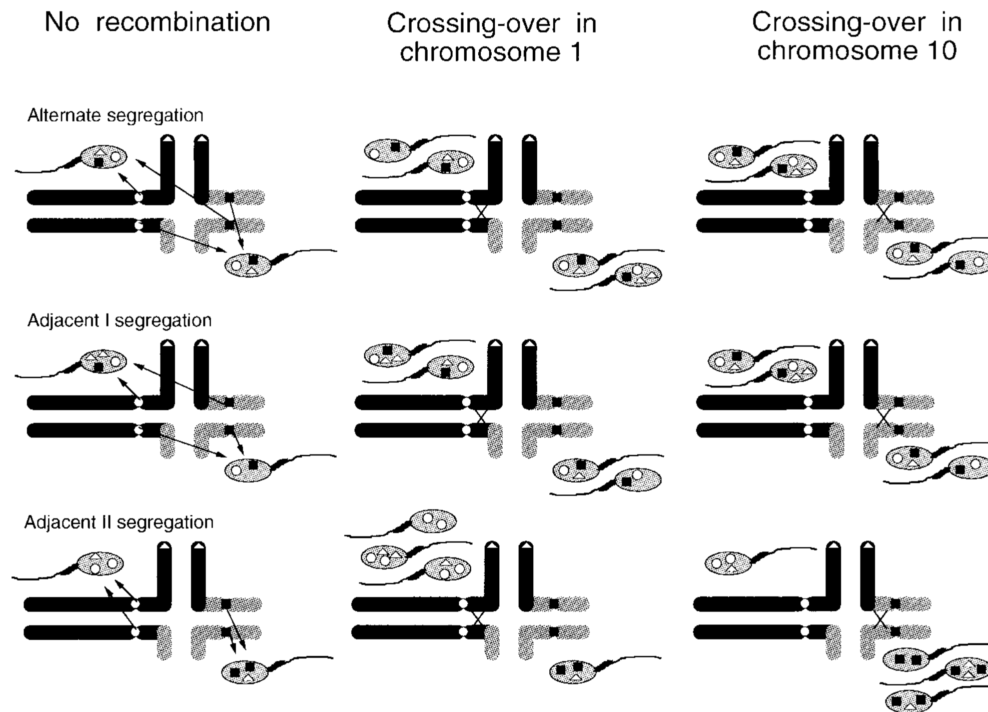

Figure 2 Illustration of the various sperm products and the presence of the probes predicted, after alternate, adjacent I, and adjacent II segregation at meiosis. The positions of the DNA probes are indicated as follows: an unblackened circle (○) for D1Z5 labeled in green, an unblackened triangle (△) for D1Z2 labeled in red, and a blackened square (■) for D10Z1 labeled in yellow.

adjacent I segregation if there was recombination between the centromere and the translocation breakpoint (interstitial segments) in chromosomes 1 or 10 or in both. Adjacent I segregation was predicted to produce two different unbalanced sperm products, 23,−10,+der(10) and 23,−1,+der(1). Their respective fluorescent phenotypes are A-M-M-10 and A-O-10, and their observed frequencies were 18.6% and 23.9%, respectively. But, if recombination in the interstitial segments had taken place, these adjacent I phenotypes also could have been produced by an alternate segregation. The frequencies of A-M-M-10 and A-O-10 sperm were predicted to be equal regardless of recombination in the interstitial segments, but A-O-10 (frequency 23.9%) was observed at significantly higher levels than A-M-M-10 (frequency 18.6%) ($P < .0001$).

Adjacent II segregation generates six different chromosomally imbalanced sperm products with distinct fluorescent phenotypes, which were observed at a total frequency of 4.9% (table 2; fig. 2). If there were no recombination in the interstitial segments, one would expect that the two reciprocal sperm products 23,−10,+der(1), or phenotype A-A-M-O, and 23,−1,+der(10), or phenotype O-M-10-10, would be produced in equal amounts. However, we observed that the reciprocal products were produced at a ratio of 1:2.8 ($P < .0001$).

The presence of the four additional and unique adjacent II products (phenotypes O-O-10-10, A-M-M-10-10, A-A-M-M-O, and A-A-O-O) indicated that recombination occurred in the interstitial segments of chromosomes 1 and 10. Sperm products from interstitial recombination were observed at significantly higher frequencies in chromosome 10 than in chromosome 1 ($P = .006$), but the frequencies of their respective reciprocal sperm products were not significantly different from the expected 1:1 ratio.

3:1 segregation was predicted to produce four specific sperm products (table 2) and were observed at a total frequency of 3.9%. Recombination in the interstitial segments could not be assessed, because this produces sperm with fluorescent phenotypes identical to those of nonrecombinants in other 3:1 segregants.

We found no evidence for nondisjunction of the entire quadrivalent complex (4:0 segregation), among 4,036 gametes. 4:0 segregation was predicted to produce two fluorescent phenotypes, with one phenotype identical to that of balanced diploid sperm (A-A-M-M-10-10) and the other to sperm not hybridized (O-O-O). The frequencies of A-A-M-M-10-10 and O-O-O sperm, therefore, should be significantly higher than the frequency of diploid sperm (0.3%) or of nonhybridized sperm (0.001%), as estimated by the four-probe aneuploidy assay (table 3), but were observed at frequencies of .1% and .02% (table 2), respectively.

Table 2

Sperm Products of Meiotic Segregation in a t(1;10)(p22.1;q22.3) Carrier, Detected by Multicolor FISH Using Probes D1Z5, D1Z2, and D10Z1

Sperm Product Type and Fluorescence Phenotype	Associated Genotype	No. of Sperm (%) [n = 4,036]
Alternate and adjacent I:		
A-M-10	Normal or 23,−1,−10,+der(1), +der(10)	1,941 (48.09)
A-M-M-10	23,−10,+der(10)	750 (18.58)
A-O-10	23,−1,+der(1)	963 (23.86)
Total		3,654 (90.54)
Adjacent II:		
A-A-M-O	23,−10,+der(1)	31 (.77)
O-M-10-10	23,−1,+der(10)	87 (2.16)
O-O-10-10	23,−1,+10	21 (.52)
O-M-M-10-10	23,−1,−10,+der(10),+der(10)	30 (.74)
A-A-M-M-O	23,−10,+1	18 (.45)
A-A-O-O	23,−10,−1,+der(1),+der(1)	9 (.22)
Total		196 (4.86)
3:1 segregation:		
A-M-10-10	24,−1,+der(1),+der(10)	17 (.42)
A-M-O	22,−10	19 (.47)
A-M-M-10-10	24,+der(10)	10 (.25)
A-O-O	22,−1,−10,+der(1)	34 (.84)
A-A-M-10	24,+der(1)	6 (.15)
O-M-10	22,−1,−10,+der(10)	45 (1.11)
A-A-M-M-10	24,−10,+der(1),+der(10)	9 (.22)
O-O-10	22,−1	19 (.47)
Total		159 (3.94)
Diploid sperm:		
A-A-M-M-10-10	Balanced diploid or 4:0 segregation	5 (.12)
A-A-M-M-M-M-10-10	46,−10,−10,+der(10),+der(10)	2 (.05)
A-A-O-10-10	46,−1,−1,+der(1),+der(1)	5 (.12)
A-A-A-A-M-M-O	46,−10,−10,+der(1),+der(1)	1 (.02)
Total		13 (.33)
Other:		
O-O-O	4:0 segregation or unhybridized	1 (.02)
A-A-M-10-10, A-M-M-M-10, 0-M-M-10, A-M-M-0, A-M-10-10-10		13 (.32)
Total		14 (.35)

Aneuploidy Frequencies of Chromosomes Not Involved in the Translocation

Table 3 compares the aneuploidy frequencies of chromosomes X, Y, 18, and 21, for the translocation carrier and for two normal controls. There were no statistically significant differences in the frequencies of autosomal and gonosomal hyperhaploidy, between the translocation carrier and the two chromosomally normal controls. The total frequency of diploid sperm in the translocation carrier (.3%) was significantly different from that of the controls (P = .008). A frequency of 0.3% diploid sperm also was estimated by the three-probe segregation assay. The latter frequency was determined by the addition of the frequencies of the A-A-M-M-10-10 phenotypes and of all the observed double phenotypes of any of the segregation products (table 2; fig. 2).

Discussion

Chromosomes involved in a reciprocal translocation typically form quadrivalents at meiosis I (Hultén 1974; Burns et al. 1986) and will segregate in various ways, to produce high frequencies of chromosomally imbalanced sperm. The most common sperm products in our translocation carrier arose from alternate and adjacent I segregation (frequency 90.5%). Products of adjacent II and 3:1 segregation were found in much lower frequencies (4.9% and 3.9%, respectively). Nondisjunction of the whole quadrivalent most probably did not occur. These findings are consistent with 31 published cases of different human reciprocal translocations analyzed by the hamster technique. This suggests that the oocyte penetration in the hamster technique is not a barrier for specific sperm genotypes, as was suggested in other stud-

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Table 3

Frequency of Hyperhaploid and Diploid Sperm Detected by the Four-Probe Aneuploidy Assay (with Chromosomes X, Y, 18, and 21) For Two Normal Controls (Donors I and A) and for the t(1;10) Translocation Carrier

Aneuploidy and Chromosome and/or Haplotype	Frequency, for (%)		
	Donor I[a]	Donor A[b]	t(1;10)[c]
Gonosomal hyperploidy:			
X-X-8-21 or Y-Y-8-21	.05	.04	.03
X-Y-18-21	.03	.07	.02
Total	.08	.11	.05
Autosomal hyperploidy:			
18; X-18-18-21 or Y-18-18-21	.02	.03	.01
21; X-18-21-21 or Y-18-21-21	.04	.06	.07
Diploidy:			
X-X-18-18-21-21 or Y-Y-18-18-21-21	.07	.07	.15
X-Y-18-18-21-21	.12	.07	.19
Total	.19	.14	.34[d]

[a] 10,034 cells were scored; sex ratio (Y/X) of .980.
[b] 10,020 cells were scored; sex ratio (Y/X) of 1.004.
[c] 10,017 cells were scored; sex ratio (Y/X) of .991.
[d] Statistically significant difference between the results for the translocation carrier and for both controls, as analyzed by a one-sample χ^2 test ($P = .008$).

ies (Sonta et al. 1991; Chayko and Martin-DeLeon 1992). It also suggests that different reciprocal translocations do not tend to segregate one type of imbalanced gamete preferentially (for a review, see Estop et al. 1995; Martin and Spriggs 1995). Therefore, it may not be imperative to screen every translocation carrier for reproductive risk. We have demonstrated, however, that multicolor FISH analysis of human sperm has enhanced our understanding of meiotic segregation, crossing-over, and aneuploidy for a man carrying a reciprocal translocation.

We observed a statistically significant distortion ($P < .0001$) from the expected 1:1 ratio, for the reciprocal sperm products of adjacent I segregation, 23,−10,+der(10) and 23,−1,+der(1). Unequal numbers of adjacent I sperm products also were present (but not statistically significant) in nine other translocation carriers analyzed by use of the hamster technique (Brandriff et al. 1986; Templado et al. 1988; Estop et al. 1992, 1995; Martin and Spriggs 1995). Postmeiotic selection against certain genotypic sperm may induce this ratio distortion, but postmeiotic selection is unlikely, since early or round spermatids are connected by intercellular bridges and are known to share gene products (Moens and Hugenholtz 1975; Grier 1976) and since gene expression is inactivated by the time spermatozoa cast off the syncytia (Kierszenbaum and Tres 1978). Otherwise, the 1:1 ratio distortion can be explained in terms of unresolved chiasmata at the end of meiosis I (see fig. 3). This may be the case for chiasmata between the translo-

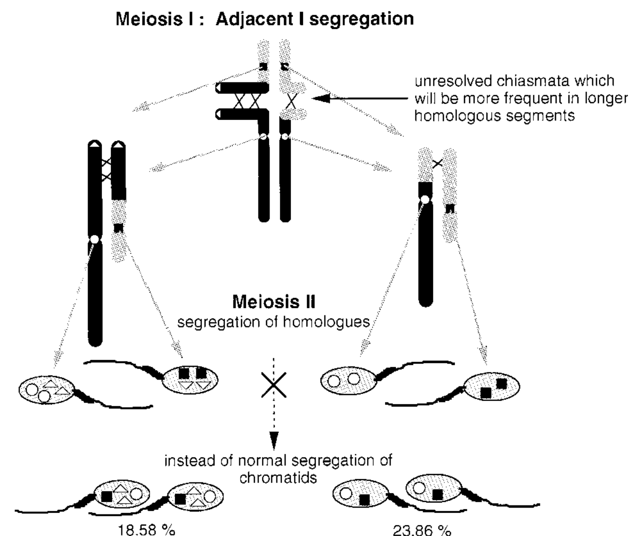

Figure 3 Illustration of sperm products predicted after segregation of homologous chromosomes, during meiosis II. After adjacent I segregation, unresolved chiasmata between the translocated segments may lead to, at meiosis II, a segregation of homologous chromosomes, producing different fluorescent sperm types than those illustrated in figure 2. If this occurs at a frequency proportional to the length of the homologous segment, then there will be an excess of the normal adjacent I sperm carrying the relatively shorter translocated segments (23,−1,+der[1] sperm, observed at 23.86%) over the reciprocal sperm products (23,−10,+der[10] sperm, observed at 18.58%). The positions of the DNA probes are indicated as follows: an unblackened circle (○) for D1Z5 labeled in green, an unblackened triangle (△) for D1Z2 labeled in red, and a blackened square (■) for D10Z1 labeled in yellow.

Table 4

Ratios of Reciprocal Sperm Products from Adjacent I Segregation in Six Different Translocation Carriers Analyzed by the Hamster Technique and in One Translocation Carrier Analyzed in This Study

Translocation Carrier	No. of Sperm Analyzed	Short/Long Translocated Genotype	Segment Ratio
t(2;18)(p21;q11.2)[a]	165	−2,+der(2)/−18,+der(18)	1.27
t(5;7)(q13;p15.1)[a]	157	−7,+der(7)/−5,+der(5)	1.28
t(1;4)(p36.1;q31.3)[b]	115	−1,+der(1)/−4,+der(4)	1.75
t(1;4)(p36.1;q31.3)[b]	90	−1,+der(1)/−4,+der(4)	1.37
t(8;15)(p22;q21)[c]	226	−8,+der(8)/−15,+der(15)	1.68
t(3;16)(p23;q21)[c]	201	−3,+der(3)/−16,+der(16)	.93
t(1;10)(p22.1;q22.3)[d]	4,036	−1,+der(1)/−10,+der(10)	1.28

NOTE.—Only studies that analyzed >90 sperm complements are included.
[a] Estop et al. 1995.
[b] Estop et al. 1992.
[c] Brandriff et al. 1986.
[d] This study.

cated segments, during adjacent I segregation, or between the nontranslocated segments, during adjacent II segregation, and the frequency of unresolved chiasmata may be proportional to the length of the homologous segments. Partial bivalents in meiosis II then may result in a segregation of the homologous chromosomes (reductional segregation) instead of the chromatids (equational segregation) (Nicklas 1977) or in a meiosis II arrest because of a disturbance of the bipolar spindle attachment (McKim and Hawley 1995). The overall consequence will be either a relative excess of sperm products carrying short translocated or short nontranslocated segments over their reciprocal products or, in the case of this study, an excess of 23,−1,+der(1) products over 23,−10,+der(10) products, in adjacent I segregation, and an excess of 23,−1,+der(10) products over 23,−10,+der(1) products, in adjacent II segregation. If this ratio distortion is a function of the length of the homologous segment, then it would be independent of the type of translocation. Table 4 shows the frequencies of adjacent I reciprocal sperm products from six different translocation carriers, from whom at least 90 sperm complements were analyzed by the hamster technique (Brandriff et al. 1986; Estop et al. 1992, 1995), and, in all but one translocation carrier (Brandriff et al. 1986), the sperm carrying the shortest translocated segments were more frequent than the reciprocal products. By comparison of the variability in the ratios with the expected variability based on binomial probability (when ratio = 1 is assumed), by χ^2 statistics, for each study, the ratio = 1 distortion was on the borderline of statistical significance ($P = .08$) but was significant ($P < .001$) when our data were included. This suggests that the unequal production of reciprocal sperm products probably is a result of different recombination frequencies for particular chromosome sites and not of a postmeiotic selection.

Our assay allowed us to evaluate recombination events in the interstitial segments. Adjacent II segregation produced sperm with six different fluorescent phenotypes, of which four were unique for recombinant sperm in the interstitial segments (see fig. 2). The three possible sperm products with recombination in the interstitial segments of chromosome 1 (phenotypes A-A-O-O, A-A-M-M-O, and A-A-M-O) and chromosome 10 (phenotypes O-O-10-10, O-M-M-10-10, and O-M-10-10) should be produced in relative ratios of 1:1:2. If one assumes that recombination occurs in both interstitial segments, the frequency of A-A-M-O should equal the sum of the frequencies of A-A-O-O and A-A-M-M-O (0.67%), and the frequency of O-M-10-10 should equal the sum of the frequencies of O-O-10-10 and O-M-M-10-10 (1.26%). The frequencies of A-A-M-O and O-M-10-10 are predicted to be higher, because they also will be produced when there is no recombination or when recombination occurs only in one chromosome. However, the observed frequencies of A-A-M-O (0.77%) and of O-M-10-10 (2.16%) are not statistically significant from the predicted frequencies of 0.67% and 1.26%, respectively, suggesting either that recombination in the interstitial segments is closely linked to adjacent II segregation or that the interstitial segments are preferential sites for chiasmata formation. Preferential recombination in the interstitial segments was observed by meiotic metaphase I analysis (Goldman and Hultén 1993*a*). We also found a significant distortion of the 1:1 ratio of the observed frequencies of A-A-M-O and O-M-10-10 (ratio 1:2.8), suggesting that, overall, the recombination in the interstitial segments of chromosome 10 was more frequent than that in chromosome 1.

One limitation of our FISH segregation-analysis method was that normal sperm could not be distinguished from balanced sperm, because all chromosomal material, and therefore all DNA probes, are present in both sperm types. Theoretically, the ratio of normal and balanced sperm is expected to be 1:1, but equal numbers of normal and balanced chromosome complements were not always produced in other translocation carriers. A significant excess of normal sperm was found by Estop et al. (1992) and by Burns et al. (1986).

Numerous studies have questioned whether the formation of quadrivalent structures may influence the correct progression of meiosis (Aurias et al. 1978; Therman 1986; Rousseaux et al. 1995). We found no evidence of increased interchromosomal aneuploidy involving chromosomes X, Y, 18, and 21. However, somewhat higher frequencies of diploid sperm (0.34%) were observed in the translocation donor, as compared with that observed in the control donors analyzed in this study (0.16%; $P = .008$) or in the controls from previous studies (range 0.03%–0.25% [Robbins et al. 1995]; range 0.01%–0.14% [Van Hummelen et al. 1996]). It has been suggested that correct chromosome pairing and recombination may function as checkpoints in meiosis (Carpenter 1994; Kleckner 1996). Because translocations form abnormal pairing figures and have changed frequency distributions of chiasmata (Goldman and Hultén 1993*a*, 1993*b*), meiosis I or meiosis II of affected cells may be arrested with the absence of cytokinesis, leading to higher levels of diploid sperm, as was observed in this study. The increase in diploid sperm raises questions about mechanisms of triploid conceptions. However, the increased frequency of diploid sperm that was observed was very low, as compared with the aneuploid sperm products, and has to be investigated in other translocation carriers.

Our three-probe FISH technique for the assessment of the frequencies of unbalanced sperm produced by translocation carriers confirms earlier findings that used the hamster technique but does not give substantially new information concerning genetic counseling for the patient's risk of fathering genetically abnormal offspring. However, our FISH approach proved to be faster and easier than the hamster technique, and, in conjunction with a new four-probe aneuploidy assay, described in this paper, it increased the basic understanding of meiosis in translocation carriers. Moreover, with the recent availability of telomeric probes for every human chromosome (Ning et al. 1996), the sperm of any translocation carrier now can be analyzed.

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