European Journal of Human Genetics (2001) 9, 395–403

www.nature.com/ejhg

ARTICLE

Meiotic segregation analysis in a t(4;8) carrier: comparison of FISH methods on sperm chromosome metaphases and interphase sperm nuclei

Maria Oliver-Bonet*[,1], Joaquima Navarro[1], Montserrat Codina-Pascual[1], Marta Carrera[2], Josep Egozcue[3] and Jordi Benet*[,1]

[1]*Unitat de Biologia, Facultat de Medicina, Departament de Biologia Cel·lular, Fisiologia i d'Immunologia, Universitat Autònoma de Barcelona, Spain;* [2]*Centro de Patología Celular, Barcelona, Spain;* [3]*Unitat de Biologia Cel·lular, Facultat de Ciències, Departament de Biologia Cel·lular, Fisiologia i d'Immunologia, Universitat Autònoma de Barcelona, Spain*

Meiotic segregation of a t(4;8)(q28;p23) translocation carrier was determined by two different methods to compare the final results. A total of 352 sperm chromosome complements, obtained after human-hamster *in vitro* fertilisation, were analysed by whole chromosome painting, and 6590 sperm heads were studied by fluorescence *in situ* hybridisation (FISH). Frequencies of alternate, adjacent I, adjacent II and 3 : 1 segregations were, for sperm chromosomes, 35.5, 33.2, 19.9 and 11.3% respectively. For sperm head analysis, results were 30.5, 28.5, 20.5 and 19.5% respectively. There were no statistically significant differences between the two methods with respect to the observed frequencies of sperm with balanced and unbalanced chromosome constitutions. Among unbalanced gametes, the methods differed only in the frequency of 3 : 1 segregation (χ^2, $P<0.0001$). Different factors that could explain this result are discussed. To determine possible interchromosomal effects, multicolour FISH was used on sperm heads. Disomy rates of sex and 18 chromosomes were higher in the translocation carrier than in the control. The differences observed were statistically significant ($P<0.0001$ for chromosomes X and 18, and $P=0.0091$ for chromosome Y). *European Journal of Human Genetics* (2001) **9,** 395–403.

Keywords: sperm chromosome; sperm nuclei; meiotic segregation; chromosome rearrangements; aneuploidy; FISH

Introduction

Balanced reciprocal translocations are the most frequent structural chromosomal reorganisation in humans. Their frequency ranges between 0.16 and 0.092% in newborns.[1–3] Male carriers of reciprocal translocations have an increased risk of pregnancy loss and chromosomally unbalanced offspring. During meiosis I, translocated chromosomes are associated as a quadrivalent. Segregation of the chromosomes involved in the quadrivalent gives rise to different frequencies of unbalanced sperm.[4,5] Statistical data from different studies show that the predominant forms of segregation in heterozygous carriers are alternate and adjacent I. However, the relative proportion of balanced and unbalanced gametes is not constant for all carriers, because the risk figures for different types of translocations depend on the configuration of the quadrivalent. This is determined by the morphology of the chromosomes involved, the length of the interstitial and translocated segments, the probability of crossing-over in the exchanged and non-exchanged segments, the location of breakpoints in G+ or G− bands,[6] and the presence or absence of large heterochromatin regions, all of which contribute to define the quadrivalent orientation and the first meiotic segregation.

To study the relative proportion of each product, two different techniques have been applied. The human–

*Correspondence: M Oliver-Bonet or J Benet, Unitat de Biologia, Facultat de Medicina, Departament de Biologia Cel·lular, Fisiologia i d'Immunologia, Universitat Autònoma de Barcelona, E-08193, Bellaterra, Spain. Tel: +34 93 581 11 75; Fax: +34 93 581 10 25;
E-mail: moliver@servet.uab.es or jordi.benet@uab.es
Received 22 December 2000; revised 6 March 2001; accepted 14 March 2001

hamster *in vitro* fertilisation[7] allows the study of the chromosome constitution of human spermatozoa using banding and FISH techniques[8] (for a review see Cifuentes *et al*[9]). Up to now, 40 translocation carriers have been analysed. This method also allows identification of the different segregation products and the structural and numerical aberrations unrelated to the reorganisation. However, the number of cells that can be analysed is quite variable, ranging from about a dozen[10] to several hundred,[11] and is limited by different factors, such as the technical skill required and the time needed to perform the study. The other technique is triple-FISH on decondensed sperm nuclei.[12] An appropriate combination of probes provides information about chromosome segregation, and also allows the study of possible interchromosomal effects, which are evaluated through an increase of the aneuploidy frequencies affecting chromosomes unrelated to the reorganisation. This technique allows the analysis of a large number of cells, although the number of probes available limits the reorganisation studies that can be performed; using this analysis it is not possible to detect other structural abnormalities that may also affect chromosomes unrelated to the translocation. Recently the segregation patterns of a translocation t(2;18) determined by triple-FISH on sperm heads have been compared by Estop *et al*[13] with the results previously reported using chromosome analysis. Due to the fact that FISH on sperm nuclei is easier to perform than the human–hamster system, this type of analysis is being used by an increasing number of centres to evaluate the risk of transmission of chromosome abnormalities to the offspring. However the differences between both procedures must be well established. In this study, we have compared the meiotic segregation patterns analysed by FISH in human sperm chromosome complements and in human decondensed sperm nuclei, in a t(4;8)(q28;p23) carrier. We have also studied the possible incidence of interstitial chiasmata on the frequencies of the different segregation patterns, and the possible interchromosomal effect on the segregation of the sex chromosomes and chromosomes 6, 18 and 21.

Materials and methods

The patient was a t(4;8)(q28;p23) translocation carrier, 31 years old at the time of the study. He was ascertained because his wife had experienced three miscarriages. One pregnancy was terminated because prenatal diagnosis revealed the presence of partial trisomies 8p23→8qter and 4q28→4qter, resulting from a 3 : 1 segregation (Figure 1).

This study was approved by the institutional ethics committee. The patient gave written informed consent.

Sperm chromosome complements were obtained using the human–hamster technique, previously described in detail,[14] with minor modifications.[8] Slides with human sperm chromosomes were stored at −20°C until hybridisation. To obtain sperm nuclei, an aliquot of frozen spermatozoa was thawed and washed in NaCl 0.9% to eliminate cryoprotectant. The sample was fixed and decondensed following Vidal *et al*.[15] Slides were maintained at −20°C until hybridisation.

All DNA probes were provided by Vysis (Vysis Inc.Downer Grove, IL, USA) and are summarised in Table 1.

Specific whole chromosome painting for chromosomes 4 and 8, centromere probe for chromosome X and satellite III

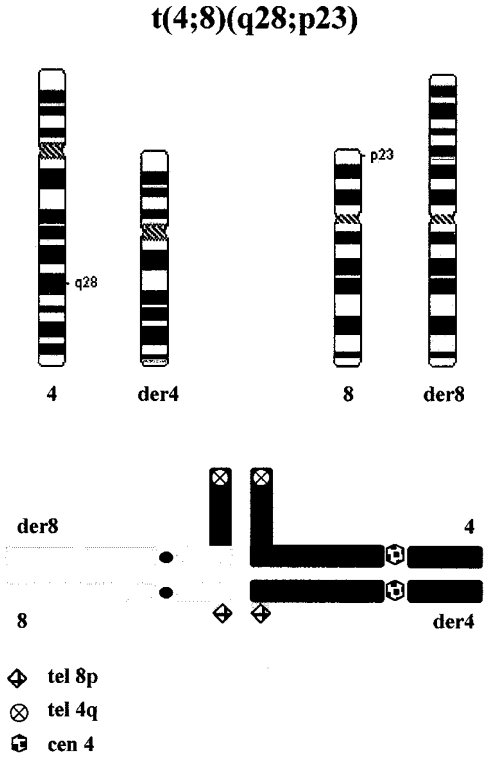

Figure 1 Ideogram and tetravalent figure at meiosis I of the translocation t(4;8)(q28;p23).

Table 1 DNA probes used for the three different analyses

Probe	*Location*	*Label*
Sperm chromosomes		
WCP 4		Spectrum Orange
WCP 8		Spectrum Green
CEP X	Alpha satellite X	Spectrum Green
DNA satellite III	Yq12	
Sperm nuclei		
CEP 4	Alpha satellite 4	Spectrum Orange +Spectrum Green
Tel 4q	4q-ter	Spectrum Orange
Tel 8p	8p-ter	Spectrum Green
Aneuploidy assay		
CEP 6	Alpha satellite 6	Spectrum Green
CEP 18	Alpha satellite 18	Spectrum Aqua
CEP X	Alpha satellite X	Spectrum Green
CEP Y	Alpha satellite Y	Spectrum Orange
LSI 21	21q22.13–21q22.2	Spectrum Orange

probe for chromosome Y were used on sperm chromosome complements. X and Y probes were used to determine the sex ratio. A probe for chromosome 18 was not used in sperm complements because the relatively low number of metaphases analysed would not allow for the detection of small differences between our laboratory control group and the translocation carrier. Frozen slides with human sperm chromosomes were thawed at room temperature. Afterwards, they were refixed and dehydrated. Five μl of probe mixture were dropped on to each slide. A coverslip was added and sealed with rubber cement. Combined denaturation was performed at 70°C for 3 min on a slide warmer. After incubation, slides were washed following manufacturer's instructions, dehydrated and counterstained with antifade (Vector lab Inc, Burlingame, CA, USA) containing DAPI at a concentration of 0.032 ng/ml (Sigma, Madrid, Spain).

Segregation analysis in decondensed sperm heads was achieved using a combination (1 : 2) of two specific probes for the centromeric region of chromosome 4, a telomeric probe for 4q and a telomeric probe for 8p.

Aneuploidy studies using two and three colour FISH were performed only on sperm nuclei, because the number of cells analysed with this method allows for the detection of small differences between the control and the translocation carrier. For the two-colour FISH analysis, centromeric and LSI autosomal probes were used to detect chromosomes 6 and 21 respectively. Three colour FISH was performed with a combination of centromeric probes for chromosomes X, Y and 18.

Hybridisation signals in both sperm chromosomes and sperm heads were observed using an Olympus Ax70 photomicroscope (Olympus Optical Co., Hamburg, Germany), equipped with four simple bandpass filters for DAPI, FITC, Cy3 and Aqua fluorescence, and a triple filter for DAPI/FITC/PI. Images were captured and produced by a Cytovision system (Applied Imaging, Sunderland, UK).

The number of red, orange and yellow signals present within the sperm head was used to determine the segregation pattern (Figure 2). Strict scoring criteria were applied. Briefly, only individual, well-delineated spermatozoa were evaluated and a sperm head was scored as having two or more signals of the same colour only when the signals were of equal size and intensity and were separated by at least one fluorescence domain.

Results

Sperm chromosomes

A total of 352 sperm chromosome complements was analysed by dual chromosome-painting. Almost all possible segregations were seen. The number of sperm chromosome complements resulting from the different segregation patterns was present at different frequencies. Numbers and percentages are shown in Table 2. Examples of each segregation pattern are presented in Figure 3a – d.

Normal and balanced forms were present in 68 (19.3%) and 57 (16.1%) cells respectively. This difference was found not to be statistically significant. Regarding adjacent I phenotypes, 59 (16.8%) of them had a normal chromosome 4 and a der(8) (23, −8, +der(8)) and 58 (16.5%) of them had a normal chromosome 8 and a der(4) (23, −4, +der(4)). A total of 70 (19.9%) chromosome complements were the result of an adjacent II segregation. Both possible adjacent II combinations produced in the absence of recombination events in the interstitial segment (23, −4, +der(8); 23, −8, +der(4)) were observed. Human metaphases with 23, −4, +der(8) (10.2%) were seen in higher percentages than metaphases with 23, −8, +der(4) (5.1%). This difference was statistically significant (χ^2, P=0.0001). If a recombination event occurs in interstitial segments, and there is an adjacent II segregation, gametes with 23, −8,+4; 23, −4, +8; 23, −4−8,+der(4), +der(4) and 23, −4, −8, +der(8), +der(8) will be produced. We have observed all of them except 23, −4, +8. Their frequencies were 1.98%, 2.3%, and 0.3% respectively.

The 3 : 1 segregation was present in 40 (11.3%) sperm complements. No significant differences were observed between the frequency of metaphases with just one chromosome involved in the reorganisation and metaphases with three different chromosomes involved in the translocation.

The sex ratio was determined in all cells and was not different from the expected 1:1 ratio.

Sperm nuclei t(4;8)

A total of 6590 spermatozoa from the t(4;8) balanced translocation carrier were analysed by triple-colour FISH with the DNA probes for CEP4, tel 4q and tel 8p. Results are summarised in Table 2. Examples of different fluorescent phenotypes are shown in Figure 4a – d.

The total number of balanced spermatozoa, with a normal or balanced phenotype (g-o-y), was found to be 2012 (30.5%). Since the probes used do not distinguish between normal and chromosomally balanced sperm, we could not detect the exact proportions of each product. Adjacent I phenotypes were present in 1876 sperm heads (28.5%); 883 (13.5%) corresponded to sperm with a 23, −4, +der(4) (g-g-y) and 993 (15.1%) to 23, −8, +der(8) sperm (o-o-y). Although both frequencies should be similar regardless of chiasmata in the interstitial segments, the observed proportions of the two types of adjacent I products were significantly different (P=0.0066). We found more frequently the products containing the long translocated segments.

The incidence of interstitial chiasmata may affect the frequencies of alternate and adjacent I segregation. Thus, in the presence of an interstitial chiasma, the g-o-y phenotype may also be generated by an adjacent I segregation, and the g-g-y and o-o-y phenotypes could result from an alternate segregation. If one exchange occurs between the centromere and the translocation breakpoint, the number of alternate g-o-y phenotypes observed should be equal to the sum of the

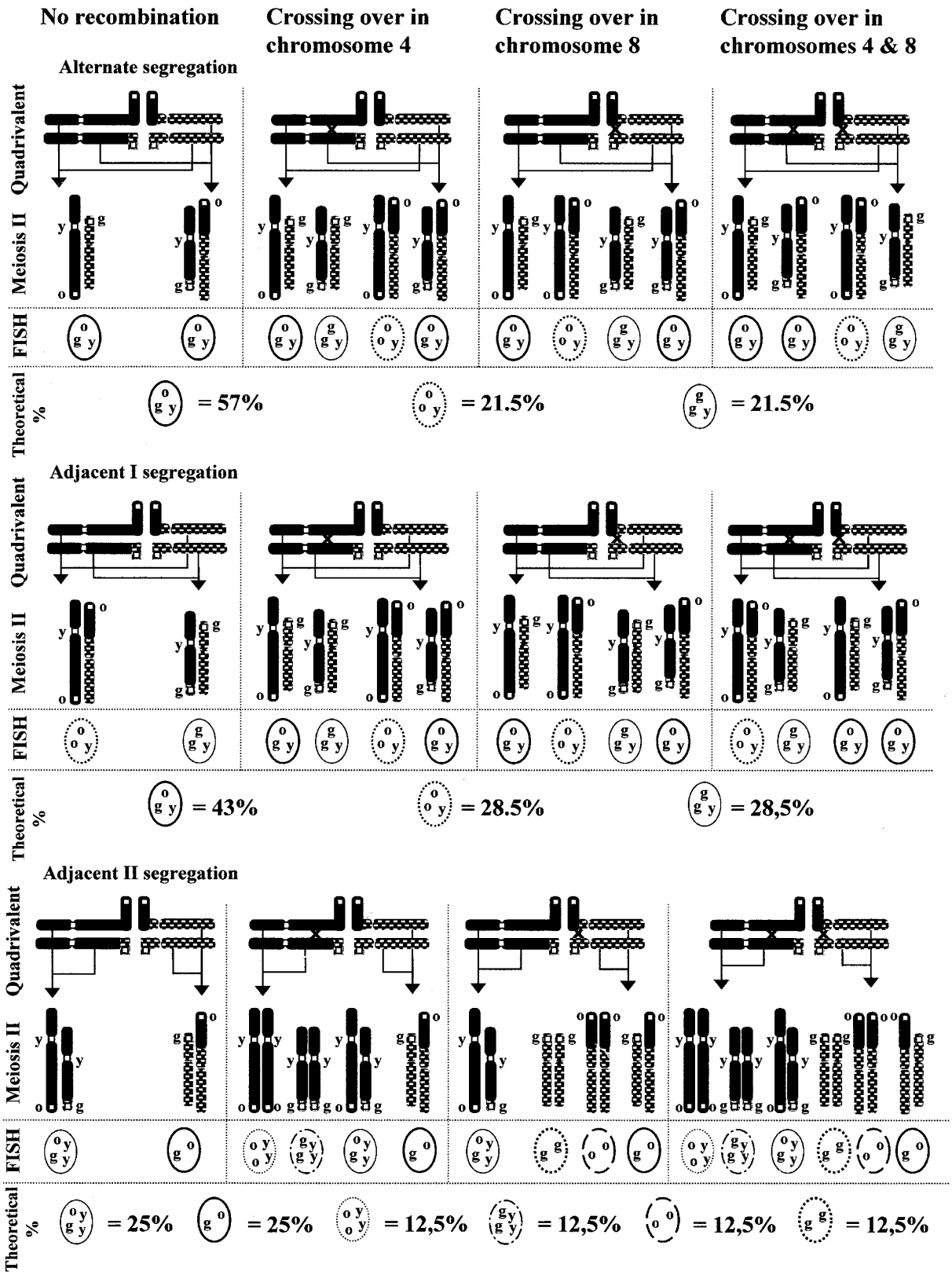

Figure 2 Meiotic segregation and fluorescent sperm head phenotypes expected, and their theoretical frequencies depending on the chiasmata formation. Fluorescent signals: *g*, green (telomere 8p), *o*, orange (telomere 4q), *y*, yellow (centromere 4).

two adjacent I phenotypes scored, thus compensating each other (see Table 2). Since we found similar phenotype frequencies of alternate and adjacent I segregation, we assumed that this compensation premise was correct. However, this assumption should only be correct if the initial number of quadrivalents that would result in an alternate segregation were equal to the initial number of quadrivalents that would produce an adjacent I segregation, which is theoretically accepted.

All adjacent II products were observed, including those resulting from recombination events within the interstitial segments of the chromosomes involved in the translocation. The total number of spermatozoa produced by adjacent II segregation was 1.351 (20.5%). The ratio of the two reciprocal adjacent II products 23, −4, +der(8), and 23, −8, +der(4) was different from the expected 1 : 1 ratio. Sperm carrying the short translocated segment (g-o) were found much more frequently (10.7%) than sperm with the reciprocal phenotype g-o-y-y (5.4%). Phenotypes resulting from interstitial chiasmata were found in 296 sperm (4.5%), and their frequencies were not significantly different from each other, regardless of the chromosomes involved in the recombination.

The 3 : 1 segregation produced eight specific fluorescent phenotypes, and was observed in 1285 sperm nuclei (19.5%). The number of nuclei with one or two signals should theoretically be equal to the number of nuclei with four or five signals, but in this study products containing just one chromosome involved in the translocation were more frequently found than those with three chromosomes. Hybridisation efficiency was 98.4%. Probes used in this study did not allow us to distinguish between 4 : 0 segregation and diploid sperm.

Aneuploidy studies of chromosomes not involved in the translocation

The aneuploidy frequencies of chromosomes X, Y, 6, 18 and 21 in the translocation carrier and in a chromosomally normal control are summarised in Table 3. Significant increases of disomy rates were observed for chromosome 18 (χ^2, $P<0.0001$) and for the sex chromosomes (χ^2, $P<0.0001$ for chromosome X and P=0.0091 for chromosome Y).

Discussion

Men heterozygous for a reciprocal translocation produce a variety of gametes, which can be either balanced or unbalanced. The study of the t(4;8)(q28;p23) carrier has shown that the frequencies of unbalanced sperm evaluated by the human – hamster technique and of sperm nuclei study

Table 2 Frequencies of different segregations in sperm and nuclei and sperm chromosomes

		Sperm nuclei	*Sperm chromosomes*
Segregation	*Flourescent phenotypes*	*Number of sperm heads (%)*	*Number of metaphases (%)*
Alternate			
n	g-o-y	2012 (30.5)	68 (19.3)
n, −4, −8+der(4),+(der8)	g-o-y		57 (16.1)
Subtotal		2012 (30.5)	125 (35.5)
Adjacent I			
n, −4,+(der4)	g-g-y	883 (13.4)	58 (16.5)
n, −8,+der(8)	o-o-y	993 (15.1)	59 (16.8)
Subtotal		1876 (28.5)	117 (33.2)
Adjacent II			
n,8,+der(4)	g-o-y-y	353 (5.4)	18 (5.1)
n, −4,+der(8)	g-o	702 (10.6)	36 (10.2)
n, −8,+4[a]	o-o-y-y	80 (1.2)	7 (1.98)
n, −4, −8,+der(4),+der(4)[a]	g-g-y-y	82 (1.2)	8 (2.3)
n, −4,+8[a]	g-g	73 (1.1)	– (–)
N, −4, −8,+der(8),+der(8)	o-o	61 (1.0)	1 (0.3)
Subtotal		1351 (20.5)	70 (19.9)
3:1			
n, −8	o-y	309 (4.7)	7 (2.0)
n, −4,+der(4),+der(8)	g-g-o-y	120 (1.8)	1 (0.3)
n, −4	g	131 (2.0)	6 (1.7)
n, −8,+der(4),+der(8)	g-o-o-y-y	62 (0.9)	6 (1.7)
n, −4, −8,+der(4)	g-y	295 (4.5)	7 (2.0)
n,+der(8)	g-o-o-y	181 (2.7)	– (–)
n, −4, −8,+der(8)	o	127 (2.0)	3 (0.8)
n,+der(4)	g-g-o-o-y	60 (0.9)	5 (1.4)
Subtotal		1285 (19.5)	40 (11.3)
Diploid or 4:0		9 (0.1)	– (–)
Others		66 (1.0)	– (–)
Total		6590 (100)	352 (100)

[a]Sperm produced by recombination in the interstitial segments of chromosomes involved in the translocation.

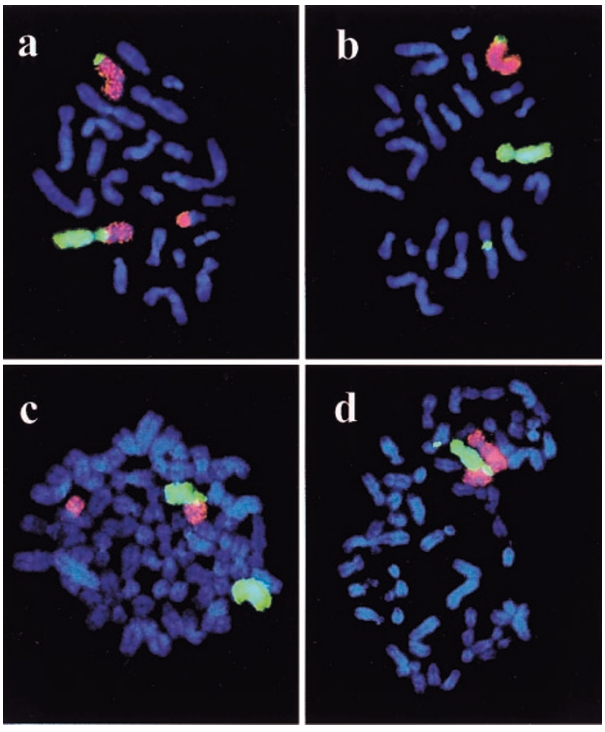

Figure 3 Sperm chromosome complements containing different segregation products. (Whole chromosome painting for chromosome 4 (red) and 8 (green), centromere signal for X chromosome (green) and satellite III for Yq12 (red)). **a**, balanced; **b**, adjacent I; **c**, adjacent II; **d**, 3 : 1 containing normal chromosomes 4 and 8, plus a translocated chromosome der(4).

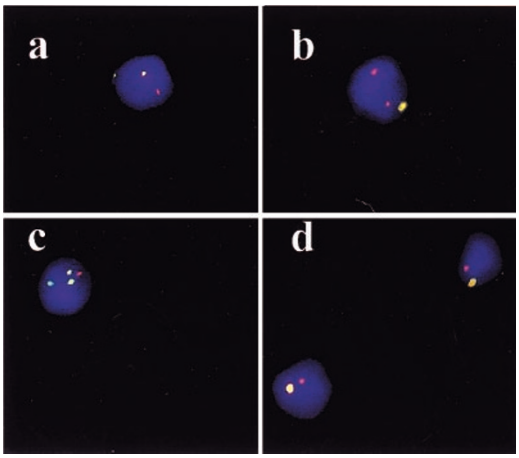

Figure 4 Sperm heads corresponding to different signal patterns. Fluorescent signals: *g*, green (telomere 8p), *o*, orange (telomere 4q), *y*, yellow (centromere 4). **a**, g-o-y, alternate; **b**, o-o-y, adjacent I; **c**, g-o-y-y, adjacent II; **d**, o-y, 3 : 1.

Table 3 Frequencies of disomic sperm

Chromosome constitution	*t(4;8) (%)*	*Control (%)*	χ^2
Normal			
X	49.7	49.7	
Y	47.1	49.3	
Gonosomal hyperploidy			
XX	0.62	0.02	$P<0.0001$
YY	0.46	0.03	$P<0.0091$
XY	0.14	0.08	
Autosomal hyperploidy			
Disomy 6	0.10	0.10	
Disomy 18	0.40	0.06	$P<0.0001$
Disomy 21	0.55	0.52	

were 64.5 and 69.2% respectively. The discordance between the information obtained from amniocentesis[16] and the rate of unbalanced sperm observed in our analysis suggests a postzygotic selection.[17] This selection may act on unbalanced zygotes, reducing their proportion during the early stages of embryogenesis because of the low viability of most unbalanced conceptions.

Sperm segregation data in translocation carriers obtained from sperm karyotyping suggests that similar proportions of balanced and unbalanced spermatozoa are produced during meiosis I. The mean frequency of both products are 46 and 54% respectively, and adjacent I segregation is the most frequent origin of unbalanced gametes.[18] Only seven of the translocation cases studied before had a higher frequency of unbalanced gametes than our patient.[18,19] In our study, the high frequency of unbalanced gametes was mainly related to a high percentage of adjacent II (19.9% in sperm metaphases and 20.7% in sperm heads) and 3 : 1 segregations (11.4 and 19.6% respectively). Similar or higher percentages of adjacent II segregations have been observed in eight reciprocal translocation carriers.[18] According to Therman and Susman,[20] adjacent II segregation will take place preferentially when either the interstitial segments or the pairing regions are so short that they do not produce chiasmata. In our case, we believe that the interstitial segment of chromosome 8 is too short to allow the formation of chiasmata. Moreover, according to Laurie and Hultén,[21] no chiasmata are expected to be found in 8p, except for a terminal one.

Regarding 3 : 1 segregation, only six translocation carriers have shown similar frequencies.[18,22] According to the hypothesis of Jalbert *et al*,[23] the following factors seem to favour a 3 : 1 over a 2 : 2 segregation: the translocated segments are very unequal in size, acrocentric chromosomes participate in the reorganisation, and at least one break is near the centromere; thus, the pachytene configuration is highly asymmetrical. In our study, the chromosome segments implicated in the reorganisation produce an asymmetrical quadrivalent (Figure 1).

When a given type of segregation occurs, the two reciprocal types of sperm phenotypes that arise from it should be found in a 1 : 1 ratio. However, in our sperm head study we have detected a significant excess of sperm carrying

the long translocated segment over sperm carrying the short translocated segment in adjacent I segregation products. Besides, an excess of sperm with the short interstitial segment over sperm with the long interstitial segment has been observed in adjacent II segregation products of both analyses. In 1997, Van Hummelen *et al*[24] explained the ratio distortion observed in adjacent I and adjacent II segregations in terms of unresolved chiasmata at meiosis I. As a result of these unresolved chiasmata, partial bivalents at meiosis II will be found, and segregation of whole chromosomes instead of chromatids will occur at anaphase II. In cases of adjacent I segregation, the frequency of unresolved chiasmata might be proportional to the length of the translocated segments; consequently, an excess of sperm with the phenotype related to the short translocated segment should be observed. In our study, the significant excess of 23, −8, +(der8) product over its reciprocal product, 23, −4, +der(4), cannot be explained by this hypothesis. Other authors have reported a similar situation.[13,25] All these cases are characterised by the presence of a very short translocated segment in one of the chromosomes involved in the reorganisation. Therefore, it does not seem improbable that other unknown factors disturb the meiotic process in addition to unresolved chiasmata.

Regarding the adjacent II frequencies observed, the excess of sperm products with short interstitial segments, 23, −4, +der(8), over sperm products with long interstitial segments, 23, −8, +der(4), can be explained by the above mentioned hypothesis of unresolved chiasmata.

Spriggs *et al*[26] and Estop *et al*[27] have compared the results of chromosome segregation into sperm nuclei by double-FISH and the results of sperm cytogenetic studies using the hamster system. Besides, Estop *et al*,[13] have reported a comparison of triple-FISH on sperm nuclei and results on sperm chromosome complements. They did not find any statistical differences between the results of both type of analysis.

We have also found a similar lack of statistical differences between the total of balanced versus unbalanced forms in the two types of analysis performed (χ^2, P=0.06). However, a statistically significant difference was found between the frequencies of 3 : 1 segregants obtained from the human–hamster technique and the 3 : 1 frequencies obtained from sperm heads (χ^2, $P<0.0001$). This significant difference could be explained by three non-mutually exclussive factors. (1) FISH on sperm nuclei is performed on smears of washed ejaculate containing dead spermatozoa as well as living, functional sperms, whereas sperm chromosome analysis requires motile sperm capable of membrane fusion and pronuclear development. A selection not directly based on the chromosomal content but on motility, carried out after a 1-h swim-up could act on sperm that, for some reason, have a reduced motility. However, since the results of Spriggs *et al*[26] and Estop *et al*[27] suggest that there is no sperm selection based on chromosome content, more comparative studies must be performed in order to settle this phenomenon. (2) FISH on sperm nuclei has higher statistical resolution than sperm chromosome complement analysis (6524 sperm nuclei versus 352 chromosome complements). However, it is important to point out that the number of chromosome complements analysed by previous comparative studies[13,26,27] ranged from 72 to 542, and no significant differences between both types of analysis were found. (3) Technical limitations of both analysis. As is well known, chromosomes can be lost during fixation with Tarkowsky's method, used in the human–hamster system. Although only well-nucleated metaphases were analysed, and metaphases with less than 22 chromosomes were discarded (even when one or more chromosomes involved in the reorganisation were present) some segregations could be misinterpreted because of the loss of one chromosome involved in the reorganisation. Thus, 24,N and 23,N chromosome complements that have lost one of these chromosomes would be misidentified as 2 : 2 and 3 : 1 segregations respectively. Although statistical differences were found in the FISH system, no significant differences between 22,N and 24,N chromosomes were observed in the human–hamster analysis (χ^2 test, P=0.4156). The excess of 3 : 1 hypohaploidies over 3 : 1 hyperhaploidies has also been observed in other two-colour FISH[26] and three-colour FISH studies.[28] Other works[22,29] indicated that technical artefacts could explain these apparent discrepancies: the sperm head is small, and as a result the number of probes that can be individually scored is limited. Even with just two probes, the possibility of superimposition of signals has to be considered. In our case, the overlapping of either red (tel4q) or green (tel8p) small telomeric signals and the yellow signal of CEP4, larger than the other two probes, could produce an apparently hypohaploid phenotype (o-y, g-y), reducing the number of sperms scored as normal or adjacent I (g-o-y, o-o-y, g-g-y) and overestimating 22,N spermatozoa with regard to 24,N spermatozoa. Another factor to consider is the unequal hybridisation efficiency observed for the probes used in this analysis. Probes for 8telp and 4telq showed fluorescence signals with an intensity lower than the CEP4 signal, probably due to their small size and also possibly to suboptimal conditions of the co-hybridisation protocol. This fact could also contribute to the misscoring of some cells. Finally, other limitations could be related to different aspects of the technique, such as the variable response of the spermatozoa from different subjects to the same treatment, the quality of the spermatozoa or an over-decondensation of the DNA that can produce split hybridisation domains and lead to apparent gains of fluorescent signals.[30]

Results obtained from the aneuploidy study suggest the possible existence of an interchromosomal effect. In translocation carriers this term refers to an increase of chromosomal abnormalities unrelated to the translocation. It has been observed in *Drosophila*,[31] in mouse,[32] and suggested in humans.[33] After the first reports suggesting an interchromo-

somal effect in humans, few data have been provided to confirm this hypothesis.[34] Some authors have reported an increased incidence of aneuploidy for chromosome 18,[35] 1[30] and 21.[36] In our study, we have found significant increases of disomy rates for chromosome 18 and for the sex chromosomes. Recently, some reports have suggested that an increase in meiotic errors in the testis could be a reflection of an abnormal testicular endocrine environment.[37,38]

Although most translocation carriers do not have abnormal levels of gonadotropins, their spermiograms are usually abnormal.[3] A possible explanation for the increases observed could be related to the asymmetry of the quadrivalent figure at pachytene. Very short translocation segments can fail to form chiasmata. As a consequence, unpaired regions will appear; it has been suggested that these unpaired regions may interfere with the synaptic process of other bivalents, producing synaptic anomalies.[39,40] These abnormalities could produce meiotic arrest, but could also lead to the production of multiple aneuploidies (reviewed by Egozcue *et al*[38]). Nevertheless, the variable frequency of aneuploidies reported among different control males reported by many authors,[41,42] and in infertile men[43] make necessary to consider our findings with caution, until the source of these variations can be clarified.

In conclusion, since the risk data for unbalanced sperm obtained by the human–hamster technique does not differ significantly from data from sperm heads analysed by FISH, both studies can be useful when information about the risk for the offspring is needed for reproductive counselling. However, our data also suggest that the use of FISH on sperm heads is not as precise as the use of FISH on chromosome complements, mainly when the signals obtained in the sperm head study can be misinterpreted for the reasons outlined above.

Acknowledgments

We thank Àngels Niubó for technical assistance. We acknowledge the financial support given by FIS, 98/0031-01 and CIRIT (1999 SGR-00104). M Oliver is the recipient of a fellowship (AP97) from the Ministerio de Educación y Cultura.

References

1 Jacobs P: Mutation rates of structural chromosomes rearrangements in man. *Am J Hum Genet* 1981; **33**: 44–54.
2 De Breakeleer M, Dao TN: Cytogenetic studies in male infertility: a review. *Hum Reprod* 1991; **6**: 245–250.
3 Van Assche E, Bonduelle M, Tournaye H *et al*: Cytogenetics of infertile men (Review). *Hum Reprod* 1996; **11** (Suppl 4): 1–26.
4 Hultén MA: Chiasma distribution at diakinesis in the normal human male. *Hereditas* 1974; **6**: 55–78.
5 Burns JP, Koduru PRK, Alonso ML, Chaganti RSK: Analysis of meiotic segregation in a man heterozygous for two reciprocal translocations using the hamster in vitro penetration system. *Am J Hum Genet* 1986; **38**: 954–964.
6 Ashley T: G-band position on meiotic synapsis and crossing-over. *Genetics* 1988; **118**: 307–317.
7 Balkan W, Martin RH: Chromosome segregation into the spermatozoa of two men heterozygous for different reciprocal translocations. *Hum Genet* 1983; **63**: 345–348.
8 Cifuentes P, Navarro J, Míguez L, Egozcue J, Benet J: Sperm segregation analysis of a complex rearrangement, 2;22;11, by whole chromosome painting. *Cytogenet Cell Genet* 1998; **82**: 204–209.
9 Cifuentes P, Navarro J, Blanco J *et al*: 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. *Eur J Hum Genet* 1999; **7**: 231–238.
10 Martin RH: Analysis of human sperm chromosome complements from a male heterozygous for a reciprocal translocation, t(11;22)(q23;q11). *Clin Genet* 1984; **25**: 357–361.
11 Spriggs EL, Martin RH, Hultén M: Sperm chromosome complements from two human reciprocal translocation heterozygotes. *Hum Genet* 1992; **88**: 447–452.
12 Blanco J, Egozcue J, Clusellas N, Vidal F: FISH on sperm heads allows the analysis of chromosome segregation and interchromosomal effects in carriers of structural rearrangements: results in a translocation carrier, t(5;8)(q33;q13). *Cytogenet Cell Genet* 1998; **83**: 275–280.
13 Estop AM, Cieply K, Wakim A, Feingold E: Meiotic products of two reciprocal translocations studied by multicolor fluorescence in situ hybridization. *Cytogenet Cell Genet* 1998; **83**: 193–198.
14 Benet J, Navarro J, Genescà A, Egozcue J, Templado C: Chromosome abnormalities in human spermatozoa after albumin or TEST-yolk capacitation. *Hum Reprod* 1991; **3**: 369–375.
15 Vidal F, Moragas M, Català V *et al*: Sephadex filtration and human serum albumin gradients do not select spermatozoa by sex chromosome: a fluorescent in situ hybridization study. *Hum Reprod* 1993; **8**: 1740–1743.
16 Daniel A, Hook EB, Wulf G: Risks of unbalanced progeny at amniocentesis to carriers of chromosome rearrangements: data from United States and Canadian laboratories. *Am J Hum Genet* 1989; **33**: 14–53.
17 Brambati B: Fate of human pregnancies; in Edwards RG (ed): *Establishing a successful Human Pregnancy*. Serono Simposia, Raven, New York, 1990, **vol 66**, pp 269–281.
18 Guttenbach M, Engel W, Schmid M: Analysis of structural chromosome abnormalities in sperm of normal men and carriers of constitutional chromosomes aberrations: A review. *Hum Genet* 1997; **100**: 1–21.
19 Estop AM, Cieply KM, Munné S, Feingold E: Multicolor fluorescence in situ hybridization analysis of the spermatozoa of a male heterozygous for a reciprocal translocation t(11;22)(q23;q11). *Hum Genet* 1999; **104**: 412–417.
20 Therman E, Susman M: *Human chromosomes: structure, behavior, and effects*. 3rd edn. Springer-Verlag, New York, 1993, pp 282–285.
21 Laurie DA, Hultén MA: Further studies on bivalent chiasma frequency in human males with normal karyotypes. *Ann Hum Genet* 1985; **49**: 189–201.
22 Martini E, von Bergh ARM, Coonen E *et al*: Detection of structural abnormalities in spermatozoa of a translocation carrier t(3;11)(q27.3;q24.3) by triple FISH. *Hum Genet* 1998; **102**: 157–165.
23 Jalbert P, Sèle B, Jalbert H: Reciprocal translocations: a way to predict the mode of imbalanced segregation by pachitene diagram drawing. *Hum Genet* 1980; **55**: 209–222.
24 Van Hummelen P, Manchester D, Lowe X, Wyrobek AJ: 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. *Am J Hum Genet* 1997; **61**: 651–659.

25 Brandriff B, Gordon L, Ashworth LK, Littman V, Watchmaker G, Carrano AV: Cytogenetics of human sperm: meiotic segregation in two translocation carriers. *Am J Hum Genet* 1986; **38**: 197–208.
26 Spriggs E, Martin R: Analysis of segregation in a human male reciprocal translocation carrier, t(1;11)(p36.3;q13.1), by two-colour fluorescence in situ hybridisation. *Mol Reprod Develop* 1994; **38**: 247–250.
27 Estop AM, Cieply KM, Aston CE: The meiotic segregation pattern of a reciprocal translocation t(10;12)(q26.1;p13.3) by fluorescence in situ hybridisation sperm analysis. *Eur J Hum Genet* 1997; **5**: 78–82.
28 Honda H, Miharu N, Ohashi Y, Honda N, Hara T, Ohama K: Analysis of segregation and aneuploidy in two reciprocal translocation carriers, t(3;9)(q26.2;q32) and t(3;9)(p25;q32), by triple-color fluorescence in situ hybridization. *Hum Genet* 1999; **105**: 428–436.
29 Rousseaux S, Chevret E, Monteil M *et al*: Meiotic segregation in males heterozygote for reciprocal translocations: analysis of sperm nuclei by two and three colour fluorescence in situ hybridization. *Cytogenet Cell Genet* 1995; **71**: 240–246.
30 Wyrobeck A, Robbins W, Mehraein Y, Weier H: Detection of sex chromosomal aneuploidies X-X, Y-Y and X-Y in human sperm using two-chromosome fluorescence in situ hybridization. *Am J Hum Genet* 1994; **53**: 1–7.
31 Grell RF: Distributive pairing in man? *Ann Génét (Paris)* 1971; **14**: 165–171.
32 Ford CE, Evans EP: Robertsonian translocation in mice: segregational irregularities in male heterozygotes and zygotic imbalance. *Chromosome Today* 1973; **4**: 387–397.
33 Lejeune J: Autosomal disorders. *Pediatrics* 1963; **32**: 326–337.
34 Serra A, Brahe C, Millington-Ward A *et al*: Pericentric inversion of chromosome 9: prevalence in 300 Down syndrome families and molecular studies of nondisjunction. *Am J Med Genet* 1990; **7** (Suppl): 162–168.
35 Mercier S, Morel F, Fellman F, Roux C: Molecular analysis of the chromosomal equipment in spermatozoa of a 46, XY, t(7;8)(q11.21;cen) carrier by using fluorescence in situ hybridization. *Hum Genet* 1998; **102**: 446–451.
36 Blanco J, Egozcue J, Vidal F: Interchromosomal effects for chromosome 21 in carriers of structural chromosome reorganizations determined by fluorescence in situ hybridization on sperm nuclei. *Hum Genet* 2000; **106**: 500–505.
37 Mroz K, Hassold TJ, Hunt PA: Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors. *Hum Reprod* 1999; **14**: 1151–1156.
38 Egozcue S, Blanco J, Vendrell JF *et al*: Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. *Hum Reprod Update* 2000; **6**: 93–105.
39 Vidal F, Navarro J, Templado C, Egozcue J: Synaptonemal complex studies in the male. *Hum Reprod* 1982; **2**: 577–581.
40 Navarro J, Vidal F, Benet J, Templado C, Marina S, Egozcue J: XY-trivalent association and synaptic anomalies in a male carrier of a Robertsonian t(13;14) translocation. *Hum Reprod* 1991; **6**: 376–381.
41 Blanco J, Egozcue J, Vidal F: Incidence of chromosome 21 disomy in human spermatozoa as determined by fluorescence in situ hybridization. *Hum Reprod* 1996; **11**: 722–726.
42 Rousseaux S, Hazzouri M, Pelletier R, Monteil M, Usson Y, Sèle B: Disomy rates for chromosomes 14 and 21 studied by fluorescent in situ hybridization in spermatozoa from three men over 60 years of age. *Mol Hum Reprod* 1998; **4**: 695–699.
43 Aran B, Blanco J, Vidal F *et al*: Screening for abnormalities of chromosomes X, Y, and 18 and for diploidy in spermatozoa from infertile men participating in an in vitro fertilization-intracytoplasmic sperm injection program. *Fertil Steril* 1999; **72**: 696–700.