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Analysis of meiotic segregation in a man heterozygous for a 13;15 Robertsonian translocation and a review of the literature

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Summary. Meiotic segregation was studied in a male heterozygous for a 13;15 Robertsonian translocation using in vitro sperm penetration of hamster eggs. Sixty-seven sperm chromosome complements were obtained and R-banded. Alternate segregation produced equal numbers of normal (31) and balanced (29) gametes, as was theoretically expected. Incidence of unbalanced complements was 10.4%, and the frequency of abnormalities unrelated to the translocation was 7.4%. This study confirms the predominance of alternate meiotic segregation in Robertsonian translocation carriers. Four sperm studies of Robertsonian translocation have been previously reported. A review of the combined results points out the low incidence of imbalance in the sperm of Robertsonian translocation carrier and the lack of evidence for an interchromosomal effect.

Introduction

With an incidence of 1/1000 live births (Evans et al. 1978), Robertsonian translocations are recognized to be the most common structural aberration in man. Among all possible types of Robertsonian translocation, the D/D class is the most frequent, and studies of both spontaneous abortions and live births indicates a high predominance of the 13;14 translocation (Hamerton et al. 1975; Jacobs 1981).

In theory, meiotic segregation in Robertsonian translocation carriers should produce similar numbers of chromosomally normal and balanced gametes (Fig. 1). Studies in a large variety of mammals have confirmed this hypothesis (Larsen et al. 1979; Logue and Harvey 1978) as have family studies in humans (Dutrillaux and Lejeune 1970; Evans et al. 1978). However in the European Collaborative Study on Prenatal Diagnosis, the frequency of balanced fetuses was higher than the frequency of normal fetuses for 13;14 Robertsonian translocations of paternal origin (Boué and Gallano 1984). Cytogenetic investigation of germ-line cells have brought forth new information about the chromosomal segregation of Robertsonian translocation. All meiotic and synaptonemal complex studies in Robertsonian translocation carriers have reported the predominance of the *cis* configuration of the translocated trivalent (Vidal et al. 1981; Rosenmann et al. 1985). Luciani et al. (1984) have suggested that this *cis* configuration favors alternate segregation (Fig. 1). At present, analysis of human sperm chromosomes provides the most direct way to study segregation patterns. Studies, using the technique of in vitro sperm penetration of golden hamster eggs, have been carried out on the sperm chromosome complements of four men heterozygous for a Robertsonian translocation. Balkan and Martin (1983) have analysed sperm from a man heterozygous for a 14;21 translocation. Pellestor et al. (1987) and Martin (1988) have studied a 13;14 translocation, and recently sperm chromosomal analysis of a t(21;22) carrier has been reported (Syme and Martin 1988). In the present study, we present the results of sperm cytogenetic analysis of a man heterozygous for the infrequently encountered 13;15 Robertsonian translocation. In a review of the literature, combined results for sperm studies of Robertsonian translocation carriers are discussed.

Materials and methods

Family report

The donor, aged 40 years and heterozygous for a Robertsonian translocation t(13;15)(p11;q11), was ascertained during investigation following the birth of a child with a trisomy 13. The mother had a normal 46,XX karotype. The birth of this child was preceded by the birth of two normal boys (4 and 7 years). Both of the donor's parents had normal karyotypes, indicating a de novo chromosome rearrangement. A pedigree is presented in Fig. 2.

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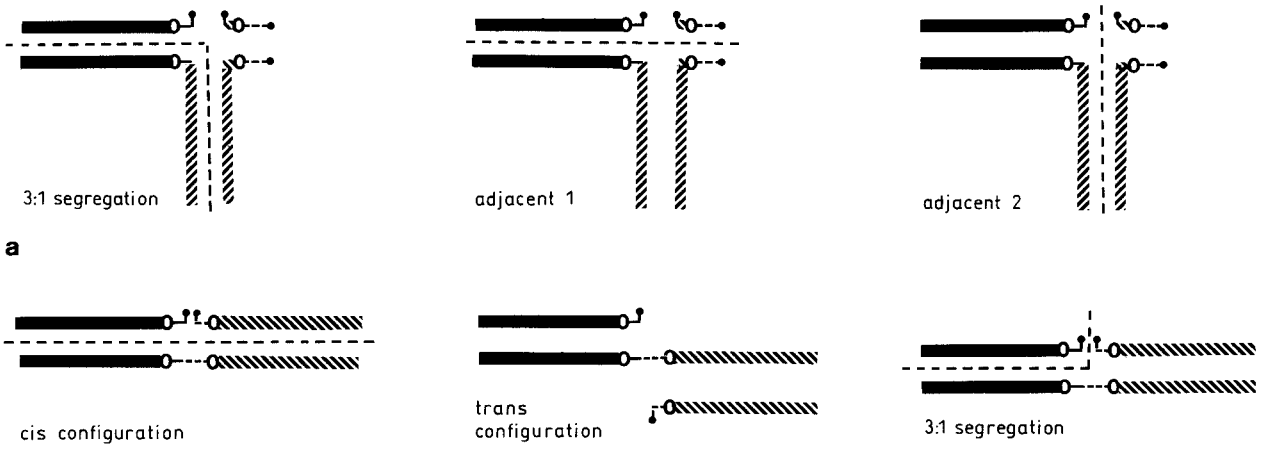

Fig. 1a, b. Meiotic segregation of D/D Robertsonian translocations. According to the type of translocation (monocentric or dicentric), two forms of pachytene conformations can occur. **a** Monocentric translocations resulting from a juxta-centromeric breakage, can be considered to be a specific type of reciprocal translocation. The pachytene arrangement can lead to four modes of meiotic segregation. Both alternate and 3:1 segregation *(left)* give normal and balanced gametes. In the 3:1 segregation, three centromeres pass to the same pole at anaphase I, but the small centric translocated fragment is often lost during later meiotic or zygotic division without phenotypical consequences. Unbalanced gametes can occur via adjacent 1 or adjacent 2 segregations. In adjacent 1 segregations (*middle*) the homologous centromeres pass to opposite poles, whereas in adjacent 2 segregations (*right*) they pass to the same pole. This results in a duplication of one translocated segment and a deficiency of the other, leading at term to a trisomy or a monosomy D. **b** In the case of a dicentric translocation two types of meiotic configuration can occur: a *cis* configuration (*left*) or a *trans* configuration (*middle*). However only the *cis* configuration has been reported in pachytene analyses of Robertsonian translocation carriers. This meiotic configuration leads to two modes of segregation: an adjacent type in which the translocated segments and the two normal acrocentrics pass to opposite poles, resulting in an equal ratio of normal and balanced cells, and a 3:1 segregation (*right*) leading at term to an imbalance, with monosomy or trisomy D

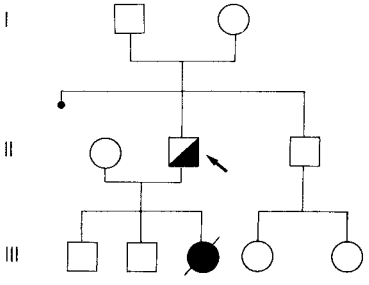

Fig. 2. Family pedigree. □ Normal male, ○ normal female, ◪ balanced translocation carrier, ● unbalanced translocation carrier, ∅ deceased, • miscarriage

Cytogenetic technique

Three semen samples were collected, all of which presented a normal spermogram (count $> 4.10^7$/ml; motility $> 50\%$). Sperm chromosome complements were obtained after sperm penetration of golden hamster eggs. The details of sperm penetration, egg collection, culture and chromosome analysis have been decribed previously (Séle et al. 1985). Haploid metaphases were systematically analysed using R-banding technique (R.H.G.).

Results

Sixty-seven haploid chromosome spreads were obtained. The results of the segregation analysis are presented in Table 1.

Table 1. Segregation of sperm chromosomes for a t(13;15) heterozygote

Segregation type	Karyotypes	No.	%
Alternate	23,X	17	25.4
	23,Y	14	20.9
	22,X,t(13;15)	16	23.9
	22,Y,t(13;15)	13	19.4
Adjacent 1	22,Y,−15 23,Y,t(13;15),+15 22,X,−15 23,X,t(13;15),+15 22,X,t(13;15),+15,−16	5	7.4
Adjacent 2	22,X,−13 23,Y,t(13;15),+13	2	3.0
Total		67	

Alternate segregation produced an equal number of normal (31) and balanced (29) gametes. An example of a balanced sperm complement is shown in Fig. 3.

The proportion of unbalanced complements was 10.4% (7/67) with 7.4% due to an adjacent 1 segregation and 3.0% resulting from an adjacent 2 segregation. The frequency of abnormalities unrelated to the translocation was 7.4% (5/67): four karyotypes were hypohaploid and one presented a double aneuploidy with a missing chromosome 8 and an extra chromosome 20 (Table 2);

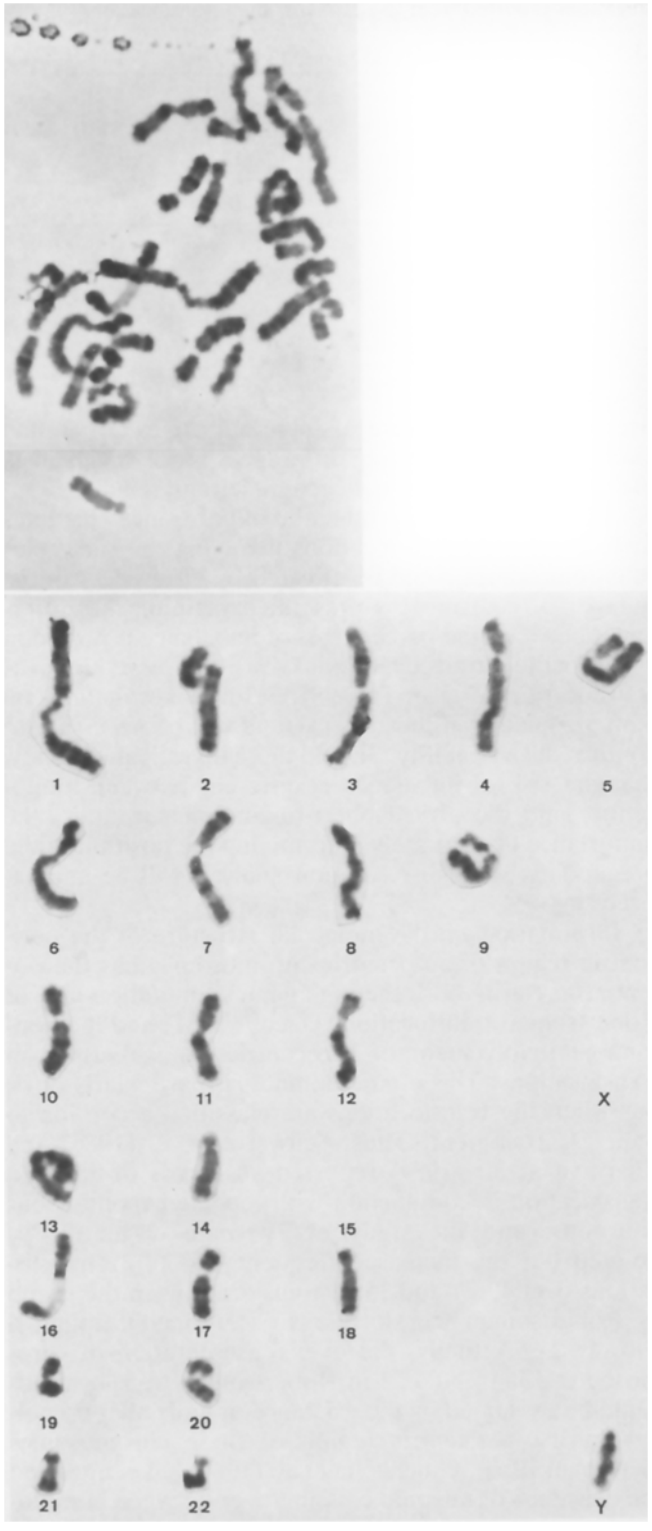

Fig. 3. An R-banded karyotype of a spermatozoa carrying the balanced form of a Robertsonian translocation 22Y,−13,−15, t(13p;15q)

no structural aberrations were found. This frequency is not significantly different ($\chi^2 = 0.27$; $P > 0.5$) from that seen in a control donor in our laboratory (9.7%). The overall sex ratio, 36X:31Y, is not different from the expected 1:1 segregation ($\chi^2 = 0{,}11$; $P > 0.5$).

Table 2. Abnormal sperm chromosome complements unrelated to the translocation

Aneuploidy	22,X,−3
	22,X,−20
	21,Y,t(13;15),−18
	22,X,t(13;15),+15,−16
Double aneuploidy	23,X,−8,+20

Discussion

In man, the 13/15 Robertsonian translocation remains an infrequent event. The few cases reported have been ascertained through trisomy 13 (Neu et al. 1973; Mori et al. 1985) or Prader-Willi syndrome (Wu et al. 1982). In the investigation of 14069 unselected newborn children, Hamerton et al. (1975) found no cases of 13/15 translocations and Cohen (1971) observed 13/15 translocations in only 9% of 64 selected individuals with Robertsonian translocations. Recently, Therman et al. (1989), in a literature review of 1266 Robertsonian translocations reported 25 cases (1.9%) of 13;15 translocations. Despite its rarity, this rearrangement is interesting because of the implication of both chromosomes 13 and 15 in chromosomal disorders. Chromosome 13 is the most often implicated of the acrocentrics in dysmorphism syndromes: retinoblastoma is associated with partial monosomy and trisomy 13, of which 20% are due to Robertsonian translocations (Cohen 1971), is well-known with a frequency of 0.07%. Chromosome 15 is involved in all chromosomal abnormalities associated with Prader-Willi syndrome. Cytogenetic banding techniques and high-resolution chromosome analysis of reciprocal translocation, Robertsonian translocation, and interstitial deletions of chromosome 15 indicate a particularly high fragility of the proximal (15q) region of the long arm of this chromosome (Mattei et al. 1984). Ledbetter et al. (1982) pointed out that when parents of Prader-Willi syndrome carriers were studied, they often had normal karyotypes, suggesting that this deletion is frequently a de novo event as is the 13;15 Robertsonian translocation reported in this report.

The meiotic segregation of the 13;15 translocation is similar to that previously reported for both a 13;14 translocation (Pellestor et al. 1987; Martin 1988) and a 21;22 translocation (Syme and Martin 1988), i.e., with an equal proportion of normal and of balanced spreads (Table 3). The only exception concerns the 14;21 translocation studied by Balkan and Martin (1983) who observed a significant excess of normal over balanced chromosome complements. It must be noted, however, that this study was based on only 24 sperm karyotypes. An equitable production of normal and balanced gametes agrees with meiotic analysis (Vidal et al. 1981; Luciani et al. 1984) and family studies (Dutrillaux and Lejeune 1970; Evans et al. 1978) of Robertsonian translocation carriers. Since normal and balanced complements result from the same type of segregation (alternate), this ratio 1:1 is expected. Thus, the excess of balanced fetuses reported for 13;14 Robertsonian translocation of pater-

Table 3. Numbers of karyotypes and frequencies (in parentheses) of segregations reported from five Robertsonian translocations

Karyotypes	(13;14)[a]	(13;14)[b]	(13;15)	(14;21)[c]	(21;22)[d]
Normal	39 (50.0)	42 (36.0)	31 (46.2)	16 (69.5)	13 (52.0)
Balanced	33 (41.3)	44 (38.0)	29 (43.2)	4 (17.5)	10 (40.0)
Unbalanced	6 (7.7)	31 (27.0)	7 (10.4)	3 (13.0)	2 (8.0)
Abnormalities unrelated to the translocation	13 (16.6)	12 (13.2)	5 (7.4)	2 (8.6)	2 (8.0)

[a] Pellestor et al. (1987)
[b] Martin (1988)
[c] Balkan and Martin (1983)
[d] Syme and Martin (1988)

nal origin in the European Collective Study of Prenatal Diagnoses (Boué and Gallano 1984) is surprising. The hypotheses of cell degeneration during spermatogenesis (Bruere et al. 1981) and prezygotic sperm selection could explain these observations, but the results of sperm analyses (Martin 1985; Pellestor and Séle 1989) argue against these theories. In fact, the data from the European study could be due to bias in the ascertainment of translocation carriers.

The frequency of unbalanced sperm observed for the 13;15 translocation was 10.4%. In the three Robertsonian translocations previously studied, this frequency ranged from 7.7% to 27.0% (Table 3), with mean of 15.8%. The incidence of imbalance in sperm from Robertsonian translocation carriers is therefore much higher than the 5.1% imbalance observed in prenatal diagnoses performed in couples in which one parent carried a Robertsonian translocation (Boué and Gallano 1984). Such a difference emphasizes the efficiency of in utero selection against imbalanced fetuses. In sperm of Robertsonian translocation carriers, all kinds of imbalances were found, whereas the majority of unbalanced fetuses reported by Boué and Gallano (1984) involved trisomy 21 (27/28 cases) suggesting a very early elimination of imbalance.

The frequency of imbalance in sperm from Robertsonian translocation carriers is lower than that observed in sperm from men heterozygous for reciprocal translocations (from 19% to 77%) (Pellestor et al. 1989). Whereas the breakpoints in Robertsonian translocations are always pericentric, reciprocal translocations can be characterized by a large variety of breakpoint positions, which result in various meiotic configurations. This variety of meiotic configurations in reciprocal translocations influences the overall percentage of imbalance, but does not affect the relative distribution of adjacent 1, adjacent 2 and 3:1 in sperm: there is always a predominance of the adjacent 1 type imbalance (Pellestor et al. 1989). The similarity of meiotic configurations in all Robertsonian translocations can explain the relatively homogeneous rate of imbalance in male Robertsonian translocation carriers. Because of the correlation between the line of chromosomal segregation and the chiasmata line (Fig. 1), there is a strong predominance of alternate segregation resulting in a low rate of unbalanced spermatozoa and consequently in a low risk of imbalance in progeny of male carriers. Thus, all imbalances in fetuses detected by prenatal diagnoses were of maternal origin (Boué and Gallano 1984), suggesting differences in the mechanism of meiotic segregation between males and females.

Modulation in the production of unbalanced gametes in Robertsonian translocations involving the same pair of chromosomes could be effected by differences in the number of centromeres present, explaining the differences between the percentages of imbalance reported in a t(13p;14q) translocation (7.4%) and a t(13q;14q) translocation (27.0%; Table 3). Future investigations on Robertsonian translocation carriers will be necessary to confirm this variability. Should these investigations show that the risk of imbalance is different between monocentric and dicentric Robertsonian translocations, the importance of accurately determining the position of the breakpoints by high-resolution analysis will be emphasized.

Information on the molecular structure of the pericentric region of acrocentric chromosomes has the potential to clarify both the high gametic mutation rate of Robertsonian translocations (Jacobs 1981) and the nonrandom involvement of acrocentrics in Robertsonian translocations. This nonrandomness is particularly obvious when the translocations are classified according to their ascertainment. Thus, Schwartz et al. (1986) and Therman et al. (1989) reported an excess of 13q;14q translocation in conjunction with repeated spontaneous abortions, and the study of Therman et al. (1989) pointed out the increased frequency of 14;21 translocations over 13;21 and 15;21 translocations in the group of Robertsonian translocations ascertained through a trisomy 21. Actually, the over-representation of chromosomes 13, 14, and 21 in Robertsonian translocations could be explained by the existence of molecular homology in the pericentric region of these chromosomes (Therman 1980). Guichaoua et al. (1986) have suggested the existence of unequal crossing-over between homologous sites of nonhomologous chromosomes, with the formation of an inverted loop. This hypothesis agrees with ultrastructural data of spatial relationships of acrocentric bivalents in nucleolar fibrillar centers (Stahl et al. 1983). Nevertheless, the low frequency of Robertsonian translocations between homologous acrocentrics indicates that other factors could be implicated in this mechanism.

An important question linked to the segregation of Robertsonian translocations concerns the existence of

an interchromosomal effect. It has been suggested that chromosomal rearrangements, particularly Robertsonian translocations, could disturb meiotic disjunction of chromosomes not involved in the rearrangement resulting in a predisposition towards trisomies (Lejeune 1965). In 1971, Mikkelsen estimated that women carrying a D/D Robertsonian translocation had a significant risk (2.0%) of having a child with trisomy 21. More recent epidemiological and family studies of Robertsonian translocation carriers disagree with the hypothesis of an interchromosomal effect: Harris et al. (1979) found no trisomic offspring in their study of the progeny of 86 carriers of 13;14 translocations, and Therman et al. (1989) indicated that chromosomes 13 and 21 are always involved in Robertsonian translocations ascertained through corresponding trisomies. Analysis of the abnormalities unrelated to the translocation in spermatozoa of men heterozygous for translocations constitutes a direct means for the investigation of the hypothesis of an interchromosomal effect. In the present study, five numerical abnormalities (7.4%) have been observed. Similar rates (from 8.0% to 16.6%) were reported to the four Robertsonian translocations previously studied (Table 3). These frequencies do not differ significantly ($P > 0.05$) from the aneuploidy rate in control donors (Pellestor and Séle 1989), indicating no evidence for an interchromosomal effect in man. Nevertheless, in a pachytene analysis of a heterozygous t(13;14) carrier, Luciani et al. (1984) have reported a preferential association between the 13–14 trivalent and the 21 bivalent, which could be related to a nonspecific effect of trivalents on the segregation of chromosome 21. In contrast to this hypothesis, most numerical abnormalities in sperm of Robertsonian translocation carriers are hypohaploidies. In fact, reports of such cases of trisomy 21 associated with translocations remain sporadic and are usually associated with reciprocal translocations (Couzin et al. 1987; Lindenbaum et al. 1985). Sperm chromosome studies performed in reciprocal translocation carriers do not show an increased frequency of abnormalities unrelated to the translocation except for the double translocation reported by Burns et al. (1986). Martin (1985) has suggested that the presence of two translocations could strongly disrupt pairing and disjunction at meiosis. Further sperm studies of translocation heterozygotes are required to accurately elucidate this important question of an interchromosomal effect.

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