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Infertility in human males with autosomal translocations: meiotic study of a 14;22 Robertsonian translocation

M. R. Guichaoua[1], B. Quack[2], R. M. Speed[3], B. Noel[2], A. C. Chandley[3], and J. M. Luciani[1]

[1] Département d'Embryologie et de Cytogénétique, Faculté de Médecine, Boulevard Pierre Dramard, F-13326 Marseille Cedex 15, France
[2] Laboratoire de Cytogénétique, F-73011 Chambéry, France
[3] MRC Human Genetics Unit, Edinburgh, UK

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Summary. Pachytene analysis was undertaken in a male patient heterozygous for a 14q22q Robertsonian translocation. The relatively low rate of XY autosome association led us to examine the relationships existing between the chromosomes involved in the translocation, the rate of XY-autosome association and the degree of spermatogenic failure. Cytogenetic investigations in infertile men and the results of the meiotic studies suggest a direct correlation between the frequency of XY-autosome association at pachytene and the degree of spermatogenic failure. Whether associations arise as a consequence or cause of germ cell failure is still not certain.

Introduction

Studies of the pachytene stage of meiosis can provide clues to the underlying mechanisms responsible for male sterility associated with some autosomal translocations (Luciani et al. 1984, 1987; Rosenmann et al. 1985; Saadallah and Hulten 1985; Chandley et al. 1986; Gabriel-Robez et al. 1986a; Johannisson et al. 1987, 1988). Three features are regularly recorded in such male-sterilizing rearrangements: (1) synaptic failure around breakpoints, (2) association of the translocation figure with the sex chromosomes, (3) frequent occurrence of an acrocentric chromosome in the translocation.

Two main models have been proposed to explain gametogenic failure in the male. Burgoyne and Baker (1984) have argued that impairment of spermatogenesis might be attributed to generalised pairing disruption around the genome, an extension of the earlier hypothesis of Miklos (1974) in which XY-pairing failure specifically was suggested as a primary cause of germ cell failure. Alternatively, the defect could result from XY-multivalent interaction, as originally proposed by Forejt (1974) for the mouse, and later suggested by Chandley (1979) to explain human spermatogenic impairment. Each mechanism may in itself be sufficient to cause gametogenic failure, but the two could also interact, partial asynapsis between the normal and translocated chromosomes favouring attraction between the translocation figure and the differential segment of the X chromosome (Rosenmann et al. 1985). In this paper, we will discuss the respective role of each mechanism in relation to the spermatogenic failure seen in an infertile male heterozygous for a 14;22 Robertsonian translocation.

Offprint requests to: J. M. Luciani

Materials and methods

The proband, a 28-year-old tin worker, married since 1981, was referred for cytogenetic investigation at the infertility clinic of the General Hospital, Chambery, France, because of primary infertility. He was phenotypically normal; height 170 cm, weight 70 kg, and his testes were normal in size. Two semen analyses carried out in the same laboratory showed oligoasthenozoospermia (sperm count: 20×10^6/ml and 15×10^6 ml; motility: 15% and 20%). Sperm morphology was normal. Mitotic chromosome preparations were made from peripheral lymphocytes cultured according to standard protocols. Testicular biopsy was performed on the left testis under general anaesthesia. Part of this material was immediately immersed in Bouin's fixative, embedded in paraffin and sectioned for pathological examination.

For light microscope pachytene analysis, meiotic preparations were obtained according to the chromomere technique of Luciani et al. (1975). The preparations were stained with Giemsa. Well-spread pachytene nuclei with preservation of the chromomeric structure of the bivalents were selected and photographed using a Zeiss photomicroscope. A small sample of the biopsy was also processed for prophase microspreading. Analysis of the pachytene configurations was made at the electron microscope (EM) level. Details of the technique can be found in Speed and Chandley (1990). Note was taken in each analysable cell of (a) the XY configuration classified according to Solari (1980) to show stage of prophase progression; (b) whether the tiny 14;22 short arms of the normal chromosomes in the trivalent were paired or unpaired and (c) whether they showed association with XY pair.

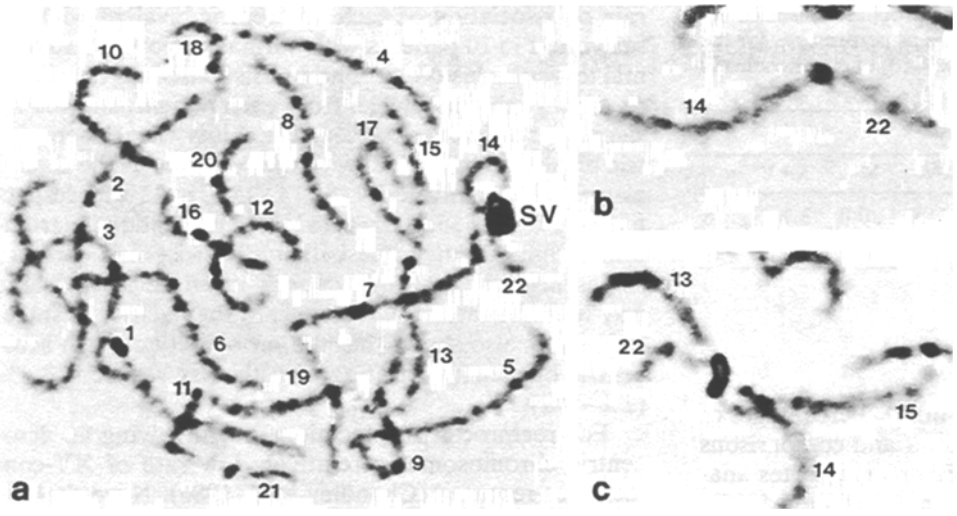

Fig. 1. a Pachytene nucleus in which all the autosomal bivalents and the 14;22 trivalent are identified by the number and sequence of their chromomeres. The trivalent is associated with the sex vesicle (*SV*) by its centromeric region, which corresponds to the breakpoints. **b, c** Details from different pachytene nuclei showing: **b** the trivalent free of association; **c** the trivalent associated with two acrocentric bivalents (13 and 15)

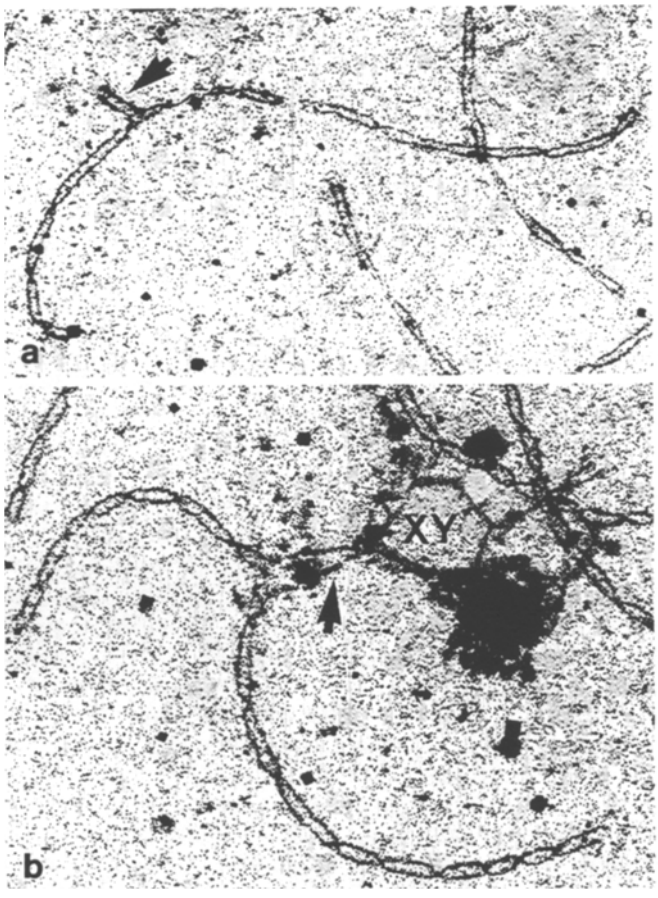

Fig. 2. a Pachytene microspread showing a fully paired trivalent, including in the short arms (*arrow*). **b** Association between the unpaired short arms of the trivalent (*arrow*) and the XY configuration (late pachytene type III-IV)

Results

Mitotic chromosome analysis

R-banding revealed the existence of a Robertsonian translocation involving chromosomes 14 and 22: 45,XY, −14, −22, +t(14;22)(p11;q11.1).

Testicular histology

Spermatogenesis was present but reduced in the majority of seminiferous tubules, few spermatozoa were present in the lumina. In some tubules, spermatogenesis was blocked at the spermatocyte or spermatid stage. There was a tendency towards reduction of the tubule diameter and thickening of the tunica propria.

Air-dried pachytene analysis

A total of 100 nuclei at the pachytene stage were selected in which the 14;22 trivalent could be unequivocally identified by the number and the sequence of chromomeres. In 28 of the 100 pachytene nuclei analysed, the trivalent configuration was closely associated with the sex vesicle in the region of the breakpoints (Fig. 1a). In 72% of nuclei, the trivalent was either free (Fig. 1b) or associated with one or more of the other acrocentric bivalents (Fig. 1c). No asynapsis within the trivalent was observed using this technique.

EM microspreading analysis

A total of 66 healthy-looking pachytene spermatocytes was analysed for the presence of the 14;22 trivalent. The number of cells available for analysis was limited because the tissue sample was delayed in transit, and on arrival in Scotland, four days after dispatch from France, many cells showed degeneration. In 28 cells (42%), fully paired short arms were observed in the trivalent, (Fig. 2a), whereas trivalents with failed pairing in the short arms were seen in 38 cells (58%). Eight such cells showed association between the unpaired (NOR-bearing) short arms and the XY configuration (Fig. 2b). XY-trivalent associations thus characterised 12% of all cells analysed by microspreading at the pachytene stage, all being found in cells showing unpaired short arms.

An analysis of 58 cells classified according to the Solari (1980) scheme, which distinguishes six progressive prophase stages between late zygotene (type 0) and late

Table 1. XY-progression through meiotic prophase classified according to Solari (1980). Figures are given as percentages for the 14;22 heterozygote and a control group ($n = 10$) of chromosomally normal men

	XY-type							
	0	I	II	III	IV	V	X+Y[a]	*n*
t(14;22)	5.2	8.6	34.5	17.2	31.0	0.0	3.4	58
Controls[b]	3.6	9.4	18.3	20.4	35.4	12.8	0.5	556

[a] Separated X and Y axes
[b] Data from Speed and Chandley (1990)

pachytene-diplotene (type V), was made. Percentages of cells of each type are given in Table 1 and comparisons are given for a control series of 556 spermatocytes analysed in a separate study (Speed and Chandley 1990). The analysis indicated a higher proportion of cells reaching the late stages of pachytene (types IV–V) in control males than in the 14;22 heterozygote, indicative of a degree of maturation impairment at the prophase stage of meiosis. Indeed, many degenerate prophase spermatocytes were found in the microspread preparations but it was not certain how many of the degenerative changes were attributable to the subfertile condition of the patient and how many to the aged state of the tissue on receipt at the Edinburgh laboratory.

Discussion

Mechanisms for XY-autosome association

In chromosomally derived sterility, some structures show contact between the rearrangement and the XY bivalent at meiotic prophase: supernumerary chromosome (Johannisson et al. 1983), Robertsonian translocations (Luciani et al. 1984; Rosenmann et al. 1985; Johannisson et al. 1987) and reciprocal translocations involving only two chromosomes (Chandley et al. 1986; Gabriel-Robez et al. 1986a; Luciani et al. 1987; Johannisson et al. 1987). This phenomenon is not significantly observed with translocations involving 3 chromosomes (Saadallah and Hulten 1985; Johannisson et al. 1988) and never observed in pericentric inversions (Guichaoua et al. 1986; Saadallah and Hulten 1986; Gabriel-Robez et al. 1986b; Chandley et al. 1987; Batanian and Hulten 1987).

From recent studies in man (Gabriel-Robez et al. 1986b; Johannisson et al. 1987), it would seem that the main condition favouring contact between a trivalent or a quadrivalent and the XY configuration at prophase of meiosis is asynapsis in one arm of the multivalent. This pairing failure is particularly noticeable in rearrangements showing acrocentric involvement and takes place between the short arms of the acrocentrics. In the present case of 14;22 translocation, non-homologous pairing of the trivalent short arms is accomplished in a great number of spermatocytes (42%) and relatively few contacts are observed. We have excluded variations of the frequency of XY-autosome association with the acrocentric chromosomes involved in the translocation by evaluating the rate of association of each acrocentric bivalent with the sex vesicle in 10 patients with normal karyotype and normal testicular histology. The results, based on the analysis of 20 pachytene nuclei from each patient, showed no significant difference between the ability of each acrocentric bivalent to associate with the sex vesicle (the rate of association for bivalents 13, 14, 15, 21, 22 was respectively 4, 7, 10, 8, 5; $X2 = 6.7$: NS). This random distribution favours the hypothesis that all acrocentric chromosomes involved in translocations interfere in the same way with the rate of XY-contact at the pachytene stage. This study also shows that the probability of each acrocentric bivalent associating with the sex vesicle is 3.4% (2%–5%).

For reciprocal translocations not involving an acrocentric chromosome, a relatively low rate of XY-contacts was reported (Chandley et al. 1986). Nevertheless, when contact did occur, it was in association with asynapsis in one small arm of the pachytene cross configuration. Studies in three – way translocations (Saadallah and Hulten 1985; Johannisson et al. 1988) gave few indications of XY-association, all arms of the hexavalents being fully paired during the pachytene stage. Extensive asynapsis around the break-points was however a feature.

These observations lend support to the belief that only when pairing is disrupted in a side arm of a translocation multivalent do contacts develop.

Factors of spermatogenic defect

Forejt (1982) and Johannisson et al. (1987) in mouse and human, respectively, attempted to demonstrate a direct correlation between the frequency of XY-autosome association and the degree of spermatogenic failure. Comparison of cytogenetic investigations in subfertile and infertile men with the results of meiotic studies leads to the same conclusions. Analysis of pooled data from four cytogenetic surveys (Marmor et al. 1980; Retief et al. 1984; Anes 1985; Bourrouillou et al. 1987; (Table 2) shows that: (1) Robertsonian translocations are more frequent than reciprocal translocations, (2) among the reciprocal translocations, four of them involve an acrocentric chromosome, (3) in patients with a sperm count below 10 million/ml, Robertsonian translocations are 2.5 times higher than reciprocal translocations (moreover, reciprocal translocations involving an acrocentric chromosome are included in this group), (4) in men with a sperm count over 10 million/ml, the number of Robertsonian translocations decreases and the ratio of acrocentric translocations/non-acrocentric translocations is inverted with regard to the previous group (0.4 versus 3.8). Thus: (1) meiotic studies suggest a high frequency of XY-association when acrocentric chromosomes are involved in the translocated figure; (2) cytogenetic investigations emphasize the severity of the spermatogenic defect when acrocentric chromosomes are involved in the translocation. The two investigations point out the similar evolution of the XY-autosome association and the impairment of spermatogenesis; this observation suggests a causal role of XY-association in spermatogenic failure. Thus, in

Table 2. Frequency of autosomal translocations with regard to the number of spermatozoa in four studies. Under the limit of 10 million spermatozoa, 48 translocations (77.42%) are found, 38 of them (61.3%) involving at least one acrocentric chromosome

Reference	Selection of patients	Total no. of patients	Total no. of autosomal translocations		No. of translocations in patients with azoospermia and sperm count < 10 *M*/ml		No. of translocations in patients with sperm count > 10 *M*/ml	
			Robertsonian	Reciprocal	Robertsonian	Reciprocal	Robertsonian	Reciprocal
Marmor et al. (1980)	Infertility and spontaneous abortion	1183	8	10	5	3[a]	3	7
Retief et al. (1984)	Sperm count < 10 *M*/ml	496	4	2	4	2[a]	–	–
Anes (1985)	Infertility	882	11	2	10	0	1	2
Bourrouillou et al. (1987)	Sperm count < 20 *M*/ml	1444	15	10	15	9[b]	0	1
	Total	4005	38	24	34 (54.8%)	14 (22.6%)	4 (6.5%)	10 (16.1%)

[a] One translocation involves one acrocentric chromosome
[b] Two translocations involve one acrocentric chromosome

our present patient, and in the 13–14 Robertsonian translocation described by Johannisson et al. (1987), the relatively low rates of association (respectively, 28% and 20%) are correlated with sperm counts over 10 million/ml.

A second hypothesis suggests that asynapsis plays a role in spermatogenic failure (Burgoyne and Baker 1984). Nevertheless, in the three way translocations described by Saadallah and Hulten (1985) and Johannisson et al. (1988), there was very little evidence of spermatogenic depression or arrest, the sperm count being within normal limits, in spite of extensive asynapsis around the breakpoints. This would argue strongly against the hypothesis of Burgoyne and Baker (1984).

Finally, a new challenge to the beliefs of Forejt (1974), Rosenmann et al. (1985) and Burgoyne and Baker (1984), has recently been put forward by Setterfield et al. (1988). They argue that synaptic failures, which can in turn lead to XY-multivalent association, arise out of poorer physiological conditions in the gonads of individuals carrying constitutional chromosome anomalies, the gametocytes having a reduced survival potential. However, why some Robertsonian rearrangements should show such high frequencies of short arm pairing disruption, and hence high frequencies of association, remains to be clarified. Genetic background will undoubtedly influence events, variability between individuals giving rise to differences in the effects that chromosomal rearrangements exert on spermatogenic development, a range of severity from near-normal to complete impairment being produced.

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