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Pachytene analysis in a 17;21 reciprocal translocation carrier: role of the acrocentric chromosomes in male sterility

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Summary. Pachytene analysis was undertaken in an infertile male, heterozygous for a 17;21 reciprocal translocation. The quadrivalent was identified by its configuration and chromomere pattern. A non-random association was found between the quadrivalent and the sex vesicle in 77% of the pachytene nuclei analysed. In 13.1% of the cells the contact with the sex vesicle was established by the terminal chromomere of the two chromosomes 21; in 63.9% of the cells, the entire region of the breakpoints was completely hidden by the sex vesicle. In some nuclei asynapsis was found in the region of the breakpoints. The nature of the contact between the quadrivalent and the sex vesicle is discussed in this paper. It is proposed that the acrocentric chromosome favours the contact between the quadrivalent and the sex vesicle, and increases the risk of sterility in male carriers of Robertsonian translocations and of reciprocal translocations involving one acrocentric chromosome.

Introduction

Recent studies of meiosis in cases of chromosomally-derived human male infertility have shown a tight association between the rearranged autosomal material and the sex chromosomes in variable proportion. The abnormal karyotypes include trisomy (Johannisson et al. 1983), Robertsonian translocations (Luciani et al. 1984a; Rosenmann et al. 1985) and reciprocal translocations (Chandley et al. 1986; Gabriel-Robez et al. 1986). The observations in man agree with the more numerous findings reported in the mouse (Forejt 1974, 1979, 1982; Forejt et al. 1981) which suggested that the failure of spermatogenic differentiation could be related to the attraction and non-homologous association between the unpaired autosomal regions of the rearranged chromosomes and the unpaired differential segments of the X and Y chromosomes.

Among the tight meiotic sex-chromosome – autosome associations so far reported in men with abnormal karyotypes [see above cited references and Holm and Rasmussen (1978), in a fertile reciprocal translocation carrier] at least one acrocentric chromosome has usually been involved.

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The present paper reports a new case of autosomal material associated with the sex vesicle at the pachytene stage in a reciprocal translocation carrier ascertained through primary sterility. Once more, an acrocentric chromosome is involved in the translocation: t(17p;21q). As acrocentric chromosomes are known to associate with the sex vesicle, we will discuss the impact of two factors on the high rate of association observed in our patient and their critical role on spermatogenic breakdown: (1) the existence of unpaired segments on the translocation tetravalent, (2) the presence of an acrocentric chromosome involved in the tetravalent.

Materials and methods

The proband, 33 years old, was referred for cytogenetic investigation because of primary infertility. His testes were normal in size. Severe oligozoospermia (sperm count 1.9 and 6 million/ml) and teratozoospermia (morphological anomalies of the acrosome and of the middle piece) were found on two successive examinations. Plasma levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone were normal (FSH, 4 mU/ml; LH, 6.50 mU/ml; testosterone 3 ng/ml). No consanguinity was found between his parents. Mitotic preparations were made from lymphocyte cultures. After thermal denaturation (Dutrillaux and Lejeune 1971), the slides were stained with Giemsa solution. Testis material was obtained by testicular biopsy under general anesthesia. Part of this material was immediately immersed in Bouin's fixative, then embedded in paraffin and sectioned for pathological examination.

For light pachytene analysis, meiotic preparations were made by using the technique of Luciani et al. (1984b). The preparations were stained with Giemsa solution and the well-spread pachytene nuclei, with preservation of the chromomeric structure of the chromosomes, were photographed using a Zeiss photomicroscope. For the visualization of synaptonemal complexes microspread spermatocytes were obtained using the method of Moses (1977). Meiotic figures spread on a grid and stained by the NOR method of Goodpasture and Bloom (1975) were selected under the light microscope and further examined under the electron microscope. The magnification was × 3000.

Results

Blood lymphocytes analysis

R-banded cells revealed the existence of a reciprocal translocation involving the short arm of a chromosome 17 and the long arm of a chromosome 21. Analysis of breakpoints indicated a karyotype 46, XY, t(17;21)(p13;q11). The family, living in Algeria, could not be examined.

Testicular histology

Sections from both testes showed normal-sized tubules with a slight increase in thickness of the tubular membrane. All stages of spermatogenesis, from spermatogonia to spermatids, could be observed in the tubules, but the proportion of dividing spermatocytes was lower than normal. The spermatogenic failure was more pronounced in the final stages, with few spermatids and rare spermatozoa formed, especially in the right testis. The interstitial tissue appeared to be normal.

Pachytene analysis with the light microscope

One hundred and twenty two nuclei at pachytene stage were selected in which the 17/21 quadrivalent could be unequivocally identified by the number and sequence of the chromomeres (Fig. 1).

In 94 of the 122 pachytene nuclei analysed (77%) the quadrivalent configuration was closely associated with the sex vesicle (Table 1). In 78 cells (63.9%), the entire region of the breakpoints was completely hidden by the sex vesicle. The remaining parts of chromosomes 17 involved in the quadrivalent, mainly their long arms, were visible, leading to identification of the translocation figure (Fig. 2a).

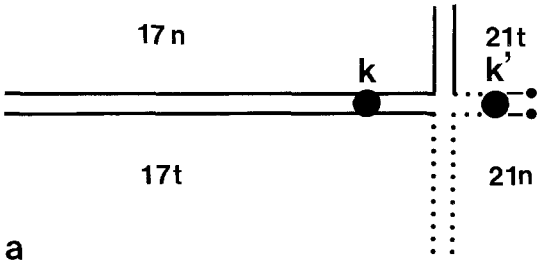
In 16 nuclei (13.1%), the tetravalent was totally visible because the contact with the sex vesicle was established by the terminal chromomere of the two chromosomes 21 (Fig. 2b). A lack of association between the sex vesicle and the quadrivalent was found in 28 pachytene nuclei (Table 1).

Asynapsis was looked for in the region of the breakpoints. Unfortunately, in 63.9% of the cells (Table 1) this region was hidden by the sex vesicle and the search proved inconclusive. However, when the breakpoint region was visible (with or without association of the quadrivalent with the sex vesicle), asynapsis was observed in 21 nuclei (Table 1). In 20 of these the asynapsis was limited to the breakpoints (Fig. 2c); in one nucleus, synaptic failure was more extended and involved the entire chromosomes 21 and the translocated region of the

Table 1. Number and percentage of pachytene nuclei showing an association between the quadrivalent and the sex vesicle

Number of pachytene nuclei analyzed	Number of nuclei with association		Number of nuclei without association	Total number of nuclei with asynapsis[a]
122	94 (77%)		28 (23%)	21 (17.2%)
	By the breakpoints region	By the terminal chromomere of chromosomes 21		
	78 (63.9%)	16 (13.1%)		

[a] Associated or not associated with the sex vesicle



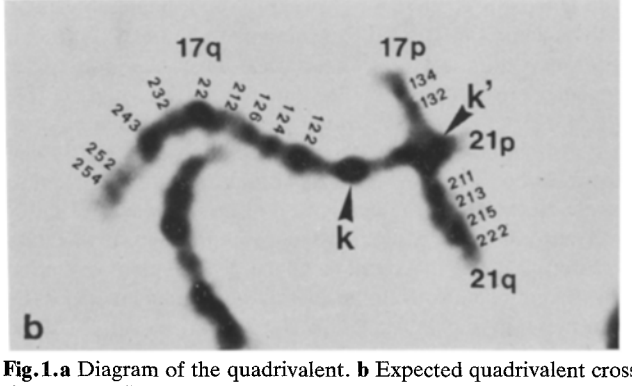


Fig. 1.a Diagram of the quadrivalent. **b** Expected quadrivalent cross-shaped configuration with determination of the breakpoints (17p13;21q11) according to the sequence of the chromomeres. k and k', centromeres of chromosomes 17 and 21 respectively

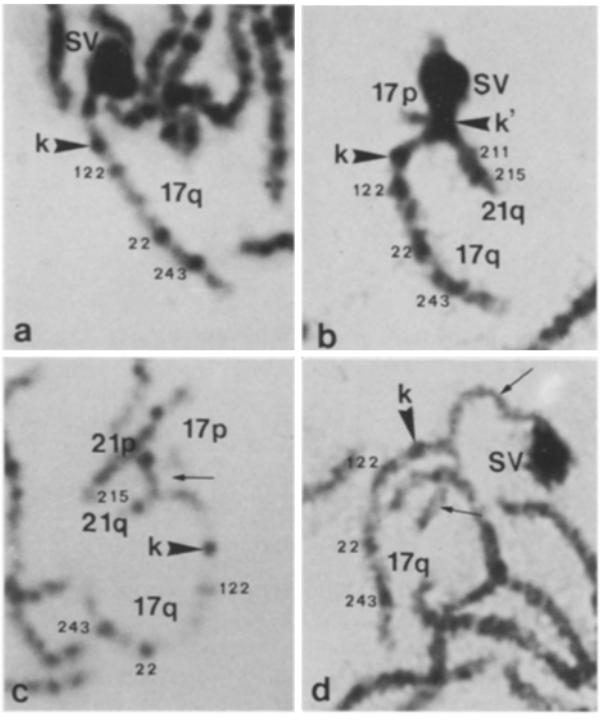


Fig. 2a-d. Details of various quadrivalent configurations and their relationships with the sex vesicle (SV). **a** The region of the breakpoints disappears behind the sex vesicle. **b** The tetravalent is connected to the sex vesicle by the terminal chromomere of the two chromosomes 21. **c** Asynapsis of the breakpoints region *(small arrow)*. **d** Extended synaptic failure involving the entire chromosome 21 and the translocated region of chromosomes 17 *(small arrows)*. Note the association of one of the asynapsed segment with the sex vesicle. (k and k', centromeres of chromosomes 17 and 21 respectively)

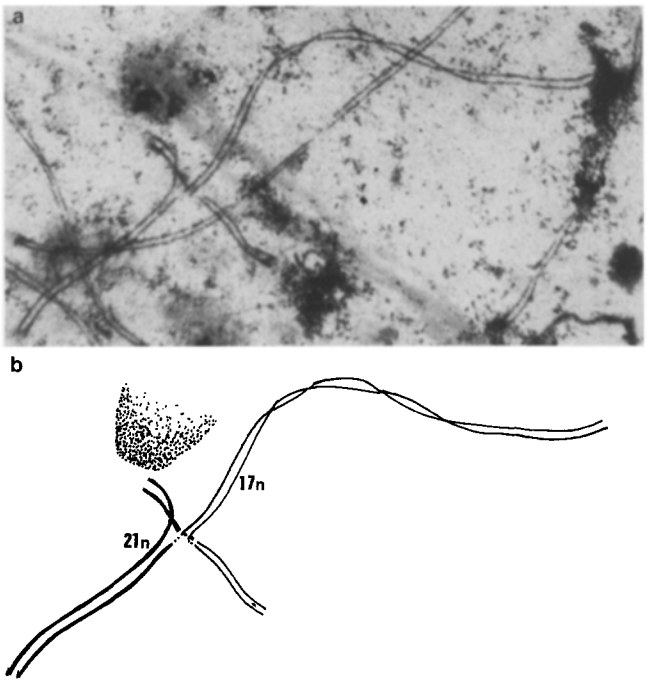


Fig. 3.a Expected cross-shaped quadrivalent configuration with a complete synapsis of normal and translocated chromosomes. **b** Interpretive drawing of the quadrivalent

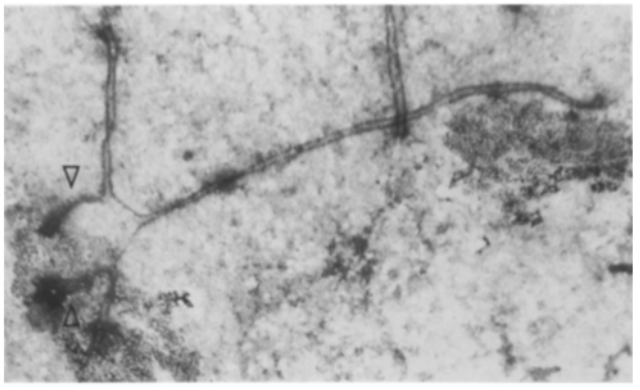


Fig. 4. Pairing among three arms of the pachytene cross, the short arms of chromosomes 21 remain non-synapsed *(open arrowheads)*

short arm of chromosomes 17 leading to the formation of free ends (Fig. 2d).

Visualization of synaptonemal complexes

Only five microspread spermatocytes were obtained in which the quadrivalent was not associated with the XY pair. Three pachytene nuclei showed complete synapsis of the normal and translocated chromosomes (Fig. 3). In two nuclei, an asynapsis of both chromosomes 21 with constitution of free ends was found (Fig. 4).

Discussion

The main purpose of our observation is to focus attention on the association of rearranged autosomal material with the sex chromosomes during meiosis and its possible role as one of the factors leading to male chromosomal infertility. We also wish to discuss the role that acrocentric chromosomes play in increasing the rate of autosome – sex-chromosome associations.

In humans, the first physical contact observed between an autosomal quadrivalent and the sex chromosomes was reported by Holm and Rasmussen (1978) in a balanced reciprocal translocation carrier 46, XY, t(5p−; 22p+). The link between sex-bivalent – autosome association and spermatogenic failure was recently demonstrated in infertile subjects, heterozygous for Robertsonian translocations 13q;14q (Luciani et al. 1984a) and 14q;21q (Rosenmann et al. 1985) and reciprocal translocations (Gabriel-Robez et al. 1986). As suggested in the mouse, both in Robertsonian and reciprocal translocations (Forejt 1979, 1982; Forejt and Gregorova 1977; Forejt et al. 1981) the XY – multivalent contact might interfere with the genetic activity of the sex chromosomes, thus resulting in metabolic disturbances and subsequent degenerative changes in germ-cell maturation.

Two types of association have been observed in our material using the light microscope. In 13.1% of the cells analysed, the whole quadrivalent was visible and the contact with the sex vesicle was through the terminal chromomere of the bivalent 21 (Fig. 2b). This pattern of association is similar to that observed in normal men whose acrocentric bivalents are known to associate with the sex vesicle (Eberle 1966; Luciani 1970; Solari and Tres 1970; Holm and Rasmussen 1977). A detailed ultrastructural description of this association has been given by Stahl et al. (1984).

The second type of association, more frequently observed (63.9%), involved the region of the breakpoints. In all studies based on visualization of synaptonemal complexes, an asynapsis was observed here (Holm and Rasmussen 1978; Rosenmann et al. 1985; Gabriel-Robez et al. 1986 and present study). This event leads to unsynapsed autosomal segments which could associate with the unpaired differential segments of the sex chromosomes. Failure of homosynapsis might lead to secondary heterosynapsis with the differential XY axes. Using serial sections, Holm and Rasmussen (1978) have shown the presence of a stretch of synaptonemal complex between a segment of the normal chromosome 5 and the long arm of the X-chromosome in a t(5–22), proving the existence of genuine pairing between the unpaired segments of the X and the asynapsed autosomal region in the vicinity of the breakpoints. Such a heterosynapsis could account for reactivation of the transcriptional activity of the X-chromosome during the first meiotic prophase in the male (De Boer and Branje 1979; Davisson et al. 1981; Forejt 1982; Johannisson et al. 1983; Luciani et al. 1984a; Rosenmann et al. 1985). Granular appearance and lateral excrescenses have been described along the autosomal segments that remain unsynapsed (De Boer and Branje 1979; Viguie et al. 1982; Johannisson et al. 1983; Rosenmann et al. 1985; Ratomponirina et al. 1985; Gabriel-Robez et al. 1986). This configuration is believed to be a characteristic of the condensed differential axes of the X and Y chromosomes at the early pachytene stage. It was then suggested that the inactivation of the XY bivalent extended from the gonosomes to the unpaired adjacent segment of the autosomes (Viguie et al. 1982; Ratomponirina et al. 1985; Gabriel-Robez et al. 1986).

Interaction between autosomal material and sex chromosomes might not be the only cause of spermatogenic breakdown. Burgoyne and Baker (1984) have suggested that a fail-

ure of synapsis gives rise to a selective destruction of meiocytes. This assumption is based on the hypothesis that the presence of unpaired meiotic sites increases the probability of spermatogenic failure as a result of an inappropriate post-meiotic transcription of genes in the unpaired segments (Miklos 1974). This hypothesis, first developed for sex chromosome pairing, was extended to the autosomes. Our present observation and recent examples of human structural aberrations, such as reciprocal translocations (Saadallah and Hulten 1985; Chandley et al. 1986) and pericentric inversions (Guichaoua et al. 1986), that show pairing difficulties and infertility could be relevant to this process.

Thus, impairment of spermatogenesis in structural chromosome abnormalities may be caused by: (1) the lack of synapsis, the unpaired autosomal segments acting through inappropriate expression of genes, (2) the physical contact between autosomes and sex chromosomes as a result of a tendency of unsynapsed segments to associate and its secondary effect on X chromosome or autosomal associated segments.

It is of added interest to notice that all the studies so far been reported with a high rate of meiotic sex-chromosome – autosome association (over 60%) show that one or two acrocentric chromosomes are involved in the translocation.

On the other hand, in the cases of non-acrocentric translocations, either no selective association was found (Saadallah and Hulten 1985: complex 2;4;9 translocation) or the rate of association was low (20%, Chandley et al. 1986, 9;20 reciprocal translocation). This last rate, however, is still significantly higher than that observed in pachytene cells prepared by microspreading from chromosomally normal individuals (Chandley et al. 1986, 4%).

Of note is the frequency of association of the multivalent with the sex vesicle (13.1%) via the terminal chromomere of the bivalent 21, constituting part of the quadrivalent. This number can be compared with the rate of association of all the acrocentric bivalents with the sex vesicle in normal males, using the same technique (9.92%, Luciani et al. 1984b). Consequently, the rate of association of the bivalent 21 when involved in the translocation quadrivalent is higher than the rate of association of the bivalent 21 by itself in normal men. The high rate of association of bivalent 21 constituting part of the quadrivalent 17; 21 can be related to the presence of asynapsis in the region of the breakpoints. Indeed, in the case reported by Chandley et al. (1986) involving two non-acrocentric chromosomes, a non-random association exists between the quadrivalent and the sex chromosomes. Analysis of their data shows that the quadrivalent 9; 20 is associated with the sex vesicle when an asynapsis is present: the more the asynapsis is extended, the higher the frequency of contact.

Hence, the two events, the presence of acrocentric chromosomes and asynapsis, could act simultaneously and additively to associate the multivalent with the sex chromosomes. This double action could explain the high rate of association found in the translocations in which one or two acrocentric chromosomes are involved.

Assuming the two hypotheses: (1) that the non-synapsed regions of the multivalent associated with the sex chromosomes cause the infertility, (2) that the presence of one or two acrocentric chromosomes favours this contact, then Robertsonian translocations can be expected to be more frequent than reciprocal translocations in a population of infertile men when compared to normal males. Pooled data from several cytogenetic surveys performed in male infertility (Zuffardi and Tiepolo 1982) confirm this theoretical expectation: Robertsonian translocations in infertile men are 10 times higher than in newborns (5.9 versus 0.6 per thousand) while the frequency of non-Robertsonian translocations is 3 per thousand versus 0.8 per thousand.

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