Pachytene Analysis in a Human Reciprocal (10;11) Translocation

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Summary. Pachytene quadrivalents are described in a male heterozygous for a balanced reciprocal translocation between the long arms of chromosomes 10 and 11. The break points of the translocation occur at 10q23 and 11q24. The main chromosome patterns of the bivalents correspond to the main G bands in mitosis and are sufficiently pronounced to allow the identification of bivalents 10 and 11 in normal spermatocytes.

In the meiotic analysis of a male heterozygous for a reciprocal translocation between the long arms of chromosomes 10 and 11, the pachytene cells were of unusual interest in that the translocation and its break points could be identified in a surprising number. The observations provide what appear to be the first illustrations of a pachytene quadrivalent in a mammal, being remarkably similar to those observed many years ago in an 8;9 translocation in maize (McClintock, 1930). They also suggest that the chromomere patterns of pachytene bivalents correspond in general to the main bands produced in mitotic chromosomes by Giemsa banding techniques, and lead to the identification of bivalents 10 and 11 in the human pachytene map.

Materials and Methods

The meiotic preparations were made from testis material obtained by biopsy from W.A., the father of a mentally retarded child who has an unbalanced karyotype, 46,XX,der(11),rcp(10;11)(q23;q24). The clinical and chromosomal findings, and the genetic marker studies in this child and her family are to be described elsewhere (Ferguson-Smith, Ellis, and Newman, in preparation). Immediately after removal, part of the testis material was placed in Waymouth's TCM + 10% calf serum containing heparin and minced with scissors (Hungerford, 1971). This material was then transferred to hypotonic 0.125 M potassium chloride for 1.25 hr at 37° C. An alternative hypotonic pretreatment in 0.12% sodium citrate for 40 min was used for part of the specimen; this produced better separation of the bivalents in diakinesis but was less successful for pachytene analysis. After fixation in 3:1 methanol-glacial acetic acid for 15 min, air dried drop preparations

were made and stained in 2% aceto-orcein for 3 minutes. Meiotic cells were examined and photographed using phase-contrast objectives. Mitotic preparations were made from lymphocyte cultures and were stained by the trypsin-Leishman technique (Seabright, 1971). Part of the testis material was fixed immediately and sectioned for pathological examination.

Results

Histological examination of sections of the testis material showed complete, active spermatogenesis without abnormality in all tubules. In particular, there was no sign of maturation arrest. Similarly, the meiotic preparations showed a normal proportion of cell types in metaphase, of which 10% were spermatogonial, 48% were in diakinesis, and 42% were in second meiotic metaphase. The translocation chromosomes could not be identified with certainty in any spermatogonial or second meiotic metaphase.

Diakinesis. The presence of a translocation was confirmed in 44 cells in diakinesis all of which showed a quadrivalent. In 41 cells, there were 22 chromosomal elements including the sex bivalent so that the karyotype could be designated MI, 22,XY,IV(10;11). In three cells the karyotype was MI, 23,X,Y,IV(10;11), this being a normal frequency of cells in which the X and Y are separate. In 64% of cells the quadrivalent appeared as a 'ring' (Fig. 1a) and in the remainder as a 'chain' (Fig. 1b). In the 44 cells the mean chiasma count was 56.9, which is two standard deviations above the normal mean of 51.11, SD 2.97, found in this labora-

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tory (Ferguson-Smith, 1972). The quadrivalent showed an average of six chiasmata.

The observation of ring quadrivalents indicates that the translocation is reciprocal, although this could not be completely established from the mitotic analysis. Given that the translocation is reciprocal,

the Giemsa banded preparations of lymphocyte mitoses (Fig. 2) show that the fragment of chromosome 11 carried by the abnormal chromosome 10 is small and most likely consists of the most distal band (11q25) and part of the adjacent band. Failure of synapsis or chiasma formation in first meiosis

FIG. 1. Diakinesis in W.A., a heterozygote for rcp(10;11)(q23; q24). (a) 'Ring' quadrivalent. MI, 22,XY,IV(10;11). (b) 'Chain' quadrivalent with asynapsis between normal chromosome 11 and translocation derivative 10. MI, 23,X,Y,IV(10;11).

FIG. 2. Chromosomes 10 and 11 from lymphocyte preparation in W.A., showing approximate positions of break points at 10q23 and 11q24. (Trypsin-Leishman.)

FIG. 3. Pachytene cells from W. A. (a) and (b). Pachytene quadrivalents in which synapsis is nearly complete in all four arms. (c) and (d). Pachytene quadrivalents with incomplete synapsis at centre of varying degrees. (e) and (f). Asynapsis of the shortest arm of the quadrivalent.

The centromeres of chromosomes 10 and 11 are indicated by long and short arrows respectively.

between this fragment and the corresponding region on the normal chromosome 11 would adequately explain the 36% of quadrivalents present as 'chains'.

Pachytene. In 100 well-fixed pachytene cells, the translocation could be identified with certainty in 31 cells. In 25 of these there was synapsis in all four arms although in only three cells was synapsis nearly complete (Figs. 3a and b). In most cells the centre of the quadrivalent was opened out to a variable extent (Figs. 3c and d) indicating failure of synapsis of differing degrees in all four arms. Complete absence of synapsis in the smallest arm of the quadrivalent was seen in six cells (Figs. 3e and f). It thus seems likely that some of the 'chain' quadrivalents identified at diakinesis are the result of asynapsis between the long arm of chromosome 11 and the abnormal chromosome 10, rather than the result of failure to form chiasmata between these regions.

A comparison of the pachytene figures and the mitotic karyotype (see Fig. 2) suggests that the break points in the reciprocal translocation occur in 10q23 and 11q24. These break points are shown in the diagram of the pachytene cross given in Fig. 4 which also indicates the positions of the main Giemsa bands as determined from the mitotic analysis. A

comparison with the examples in Fig. 3 (particularly 3a and 3c) shows that the extent and location of the largest pachytene chromomeres correspond well with the main Giemsa bands. The centric chromomeres and proximal long arm chromomeres of bivalent 10 which correspond to the centric heterochromatin and band q21 of mitotic 10 are particularly prominent. The dense and compact chromomeres in the long arm of bivalent 11 likewise correspond to the large positive bands q14 and q22 of mitotic 11, and the paracentric chromomeres of bivalent 11 seem to be in a position appropriate to band 11q12 (see Fig. 3c).

The chromomere patterns in the pachytene quadrivalent appear to be sufficiently consistent to allow the identification of bivalents 10 and 11 in normal cells. An attempt is made to demonstrate the degree of consistency in chromomere pattern between cells and individuals in Fig. 5 which shows a selection of bivalents identified as 10 and 11 from individuals with normal spermatogenesis.

Discussion

The pachytene analysis is of interest in the present case because it unequivocally demonstrates the nature of the translocation and indicates the relative lengths of the fragments which have been exchanged. This allows a much more accurate estimation of the break points than would have been possible by analysis of the G-banded preparations alone. Although it has been suggested from studies of similar human chromosome aberrations that some translocations between non-acrocentric chromosomes are terminal and not reciprocal (Francke, 1972), our findings show that this example is clearly reciprocal.

It seems, however, that the major implications of this case concern the identification of individual pachytene bivalents and the ultimate construction of a human pachytene map. It is possible to make a rough karyotype of a cell in pachytene based solely on size and centromere position, but more specific identification depends on other factors. Apart from the XY sex bivalent, which is easily identified by its precocious condensation, the first autosomal bivalents to be identified correctly in pachytene were the three large and two small nucleolar bivalents which corresponded to the five pairs of satellited chromosomes (Ferguson-Smith, 1964; Bordjadze and Prokofieva-Belgovskaya, 1971; Hungerford, La Badie, and Balaban, 1971a; Hungerford *et al*, 1971b). These were recognized by their terminal nucleoli, and by their tendency to cooperate with one another in forming common nucleoli, a phenomenon which may be similar to satellite association in mitosis. Trisomy can also be

FIG. 4. Diagram of the pachytene quadrivalent expected on the assumptions that the bivalent lengths are equivalent to the mitotic lengths and the break points occur at 10q23 and 11q24. Shaded areas indicate the main mitotic G bands.

FIG. 5. A selection of bivalents identified as 10 (top row) and 11 (bottom row) taken from pachytene cells from individuals with normal spermatogenesis. Mitotic chromosomes at twice the magnification are shown on the left separated from the pachytene bivalents by a tentative pachytene chromomere map. Centric heterochromatin is indicated by broken lines and main bands and chromomeres by dotted lines.

used to identify pachytene bivalents and Hungerford *et al* (1970) have shown in an adult male with mosaic Down's syndrome that the smallest of the nucleolar bivalents is the one trisomic in Down's syndrome. We have demonstrated that gross degrees of heteromorphism involving the polymorphic regions of chromosomes 9 and 16 are characteristic enough at pachytene to enable bivalents 9 and 16 to be identified (Ferguson-Smith, 1972; Page, 1973). Bobrow, Madan, and Pearson (1972) demonstrated yet another method, when they found that a Giemsa dye at pH 11.0 stained the paracentric heterochromatin of chromosome 9 a striking magenta colour both in mitosis and meiosis.

The study reported here seems to be the first in which two pachytene bivalents have been identified in terms of the mitotic karyotype through their involvement in a translocation. Moreover, the chromomere patterns of the two bivalents involved in the pachytene quadrivalent are characteristic enough to allow the identification of the same two bivalents in individuals who do not carry the translocation. An unexpected observation was that the chromomere patterns of bivalents 10 and 11 corresponded to a

considerable degree with the patterns produced in mitotic chromosomes by Giemsa banding. If this proves in general to be the case for all bivalents, an accurate human pachytene map should soon be achieved. The findings also suggest that positive Giemsa bands represent chromosomal regions which contain more densely compacted DNA.

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