Ring XY bivalent: a new phenomenon at metaphase I of meiosis in man

ANN C CHANDLEY, T B HARGREAVE*, S McBEATH,
A R MITCHELL, AND R M SPEED

*From the MRC Clinical and Population Cytogenetics Unit, and *the University of Edinburgh Department of Surgery (Urology), Western General Hospital, Edinburgh EH4 2XU.*

SUMMARY The unusual appearance of a ring XY bivalent at metaphase I of meiosis is reported in some cells of an oligospermic human male. Higher than usual frequencies of ring configuration in the XY pair were also observed during prophase I. The defect could be attributable to loss of some DNA sequences from the distal heterochromatic tip of the Y chromosome long arm.

A meiotic phenomenon which, as far as we are aware, has never previously been reported in man was recorded recently in the metaphase I primary spermatocytes of a male infertility patient. The sex bivalent, instead of appearing in its usual chain-like form with end to end association of the X and Y chromosome short arms, was seen in about 5% of cells to make a ring; secondary association was occurring also at the long arm distal tips. Such associations are a fairly regular feature of the XY pair at early pachytene in man,¹ but by meiotic metaphase I the situation is normally resolved and the sex chromosomes appear held together in tandem only by their short arm distal tips. Not only were ring XY bivalents seen at metaphase I in this unusual subject, but at the pachytene stage a much higher than normal frequency of ring configurations in the sex pair was also found.

To investigate whether a defect existed in the distal tip of the Y chromosome long arm, meiotic preparations were examined by *in situ* hybridisation using the cloned probe pHY 2-1. The data could indicate loss of DNA sequences from the heterochromatic tip of Yq although other explanations are offered.

Case report

The patient (WSM 175), a 23 year old phenotypically normal male, presented at the Infertility Clinic of the Western General Hospital, Edinburgh in April 1984. He was a healthy man employed as a labourer, who reported he had suffered no serious adult illnesses. Height was 170 cm and weight 85.3 kg.

Received for publication 16 September 1985.

Revised version accepted for publication 1 November 1985.

Both testes were of normal size and consistency and the penis and scrotum were normal.

SEMINAL ANALYSIS

A single semen sample, taken when the patient first reported at the clinic, showed severe oligospermia, the sperm count being only 1×10^6 per ml (morphology 55% normal, motility 80% normal).

TESTICULAR HISTOLOGY

A testicular biopsy, taken in October 1984, showed a very variegated histological appearance in the seminiferous tubules. Some lobules of cystic parenchyma appeared to consist solely of extensively hyalinised tubules. These were of small calibre and showed either complete absence of germinal epithelium or a markedly degenerate epithelial lining consisting of a few spermatogonia or Sertoli cells only. The tunica propria of these tubules was thickened.

Other larger tubules occupied most of the biopsy, these being lined by a normal germinal epithelium which in about 60% of cases showed normal spermatogenesis with mature spermatozoa. Even in these, however, there was evidence of maturation arrest at the secondary spermatocyte level. Other large tubules were distended and showed marked compression of their germinal component. Leydig cells were more prominent than usual.

Methods

CYTOGENETIC ANALYSIS

Somatic chromosomes

Chromosome analysis on peripheral blood lympho-

cytes of WSM 175 was performed using standard techniques. G banding,² C banding,³ and kinetochore staining⁴ techniques were all applied.

Meiotic chromosomes

Metaphase I. Analysis of metaphase I divisions was performed on conventional air dried preparations⁵ stained with carbol fuchsin. For unambiguous identification of the ring XY bivalent, prefluorescence using distamycin A/DAPI⁶ was performed on additional slides. Cells in which a ring XY was identified by fluorescence were photographed, relocated after carbol fuchsin staining, and rephotographed.

Pachytene analysis. Microspread preparations of pachytene spermatocytes were made on Parlodion coated slides, stained with silver nitrate,⁷ and examined at the electron microscope level. Spreads were first selected in the light microscope and cut from the film using a Leitz diamond slide marker. The small round discs were floated off the slides onto distilled water and picked up on G200 HsCu (Gilder) grids. Grids were examined with a Philips EM 300 at 60 kV.

IN SITU HYBRIDISATION

Air dried meiotic metaphase I cells from WSM 175 and a chromosomally normal control (WSM 179), ascertained two and a half months later, were probed using the cloned probe pHY 2.1. All slides were stored in a desiccator under vacuum at 4°C until being used for the experiment. This sequence is located in the most distal region of Yq1.2.⁸ Hybridisation in situ was carried out as described previously.⁹⁻¹¹ Slides were developed after one, two, three, four, 25, and 40 days exposure and the chromosomes were stained through the emulsion using Wright's stain.⁹ To allow an impartial analysis, each slide was coded and scored blind by a single observer.

Results

BLOOD LYMPHOCYTE CHROMOSOME ANALYSIS

A total of 10 G banded cells from WSM 175 was analysed. All showed a normal 46,XY karyotype. The Y chromosome was normal in appearance and well within normal size limits (G group size range). Additional cells analysed by C banding and kinetochore staining showed the Y chromosome to be apparently normal in structure. A scan of all suitable metaphases on one slide ($n > 100$) gave no indication of any association between the Y chromosome and other chromosomes in the complement.

The Y chromosome of the control male WSM 179

was also normal in appearance and size (G group range).

MEIOTIC CHROMOSOME ANALYSIS

Air dried preparations

A normal meiotic picture was present in this patient, other than the occurrence of the ring XY bivalent in some cells at metaphase I (MI). A distribution count of 300 dividing cells in spermatogonial mitosis, MI, and MII showed proportions well within normal limits. The frequency of univalent X and Y chromosomes (6.8%) was also within normal limits, and chiasma counts made on 10 cells at MI gave normal mean value of 53.4 chiasmata per cell.

The only exceptional feature at meiosis, therefore, was the ring XY bivalent which was first noted in a clear, well spread MI cell, stained with carbol fuchsin, and showing 23 bivalents but absence of a normal chain XY bivalent (fig 1). Subsequent analysis revealed that a total of 13 out of 268 (4.9%) MI cells in these preparations, all with normal counts of 23, lacked a chain XY bivalent. Unambiguous identification of the ring XY bivalent in a cell prefluoresced using distamycin A/DAPI is shown in fig 2. A single fluorescent segment corresponding to Yqh can be seen in one ring bivalent.

Quite fortuitously, meiotic air dried slides of WSM 175 had been used in an in situ hybridisation experiment unconnected with the present investigation.¹¹ Here, the highly repeated cloned probe

FIG 1 Air dried meiotic metaphase I stained with carbol fuchsin. The presumed XY bivalent is arrowed.

FIG 2 MI stained consecutively with (a) distamycin A/DAPI and (b) carbol fuchsin. The ring XY bivalent is arrowed.

FIG 3 *In situ* hybridisation of cloned probe Xb_1 to MI cell of WSM 175. The single heavy signal over the Yq heterochromatin in one ring bivalent identifies it as the ring XY. Heavy labelling also characterises bivalent No 9.

Xb_1 , which hybridises to the long arm heterochromatin of the Y chromosome (as well as to the secondary constriction of chromosome 9), had been used. Cells containing the ring XY bivalent clearly showed a single hot spot of label over Yqh by which the sex bivalent could be identified (fig 3).

Pachytene analysis

Among 50 pachytene cells in which the XY pair could be classified according to the types O to V of Solari,¹² in addition to the usual association at the short arms where a synaptonemal complex occurs, secondary association between the long arm tips was found in 81% of early to mid-pachytene nuclei (types I and II). This compares with the usual frequency of long arm/long arm association of about 50% recorded in our own laboratory at these stages for chromosomally normal persons.¹

A very high proportion (70%) of all pachytenes examined at electron microscope level by spreading were degenerate looking, heavily deposited with

FIG 4 (a) Whole pachytene cell from EM spread preparation. Heavy deposits of silver positive material are seen scattered within the nucleus (arrows). (b) The XY pair at pachytene in a spread preparation (EM). Heavy deposits of silver positive material are present on the differential axes.

silver positive NOR-like material, and showed poorly defined synaptonemal complexes (fig 4). We concluded that such cells were destined for maturation breakdown and would contribute significantly to the severe oligospermia of the patient. Maturation arrest of a proportion of cells was indeed noted, from the histological report, to be occurring at the secondary spermatocyte levels. The XY differential segments in many cells were also seen in this patient to be more than usually deposited with silver positive material or excrescences (fig 4b), but the significance of this in relation to the ring XY formation is not clear.

In situ hybridisation

Hybridisation of the probe pHY 2.1 to the Y chromosome at meiosis in WSM 179 is shown in fig 5, following a one day exposure to autoradiographic film. At this short exposure time, individual grains were countable in most cells from the two patients, but as exposure times increased so grains tended to coalesce and become uncountable in an increasing proportion of cells.

Grain count results from WSM 175 and WSM 179, developed after one, two, three, and four days of exposure are presented as a histogram in fig 6. Background counts for both patients were negligible. Statistical analysis using the Mann-Whitney U test showed that the difference in total grains recorded between the patient (WSM 175) and his control (WSM 179) was highly significant. Values of p were

FIG 5 *In situ* hybridisation of the cloned probe pHY 2.1 to the distal tip of Yqh at MI in the control WSM 179. One day exposure.

FIG 6 Histogram showing grain counts over the Yq heterochromatin after *in situ* labelling. Exposure times 1, 2, 3, and 4 days for patient (WSM 175) and control (WSM 179).

0.001, 0.69, 0.001, and 0.00001 for days 1, 2, 3, and 4 respectively.

After an exposure period of 40 days, the distal heterochromatin of Yq was extremely heavily labelled both in WSM 175 and WSM 179. By this time some autosomes were also becoming clearly labelled. No secondary site of label was seen, however, on the XY bivalent in either patient, in spite of examining a considerable number (>80) of MI plates on each of the 40 day exposure slides.

Discussion

Patient WSM 175 appears to be a very rare subject. In no other male has the senior author (ACC) observed a ring XY bivalent in a primary spermatocyte at metaphase I, and this is over a period of 16 years of observation on human male meiotic material, some 800 biopsy specimens processed, and several thousands of metaphase I divisions analysed. Moreover, no previous report of such an unusual phenomenon appears to have been published.

Our results using *in situ* hybridisation could indicate that the Y chromosome of WSM 175, the ring XY subject, contains fewer copies of the pHY

2.1 DNA sequences than the Y chromosome of a normal male. Previous studies¹³ have shown that Y chromosome variants lacking part or all of the fluorescent region of the long arm of the Y chromosome contain fewer copies of the repeated DNA sequences represented by probe pHY 2.1. One interpretation of the data, therefore, could be that the Y chromosome of WSM 175 is a variant which has lost chromatin containing at least a proportion of the pHY 2.1 probe DNA sequences. The loss in this case, however, was imperceptible cytologically.

It is also possible that the lower grain counts obtained for WSM 175 could be due simply to between patient or between preparation variability. In situ hybridisation is notoriously variable as a technique, with variation in grain counts occurring between different sets of slides. The original air dried meiotic preparations used in the present investigation were made with a 2.5 month interval separating them, and the different levels of hybridisation obtained could be attributable to this fact. Variation in hybridisation could also occur between individual men with normal looking Y chromosomes, even if slides were prepared at the same time. It is of interest, nevertheless, to ask if there could be a correlation between the rare event of finding a ring XY bivalent and loss of chromatin containing a repeat DNA sequence localised in the telomeric region of the Y chromosome. One explanation could be that a translocation event has occurred between Yq and Xq. Although the ring XY was noted in approximately 80% of the cells in pachytene, only 5% of the rings remained in MI. Those bivalents of WSM 175 which demonstrated a chain configuration in MI (95% of the cells) did not show any labelling of Xq. Thus, we would rule out the possibility that a translocation event had taken place.

Alternatively, an intercalary deletion within the fluorescent region of the long arm of the Y chromosome, with the specific telomere sequence undergoing translocation to the now deleted portion of the Y chromosome, may have occurred. We know that telomeres are important for DNA replication¹⁴ and models explaining their role in this procedure have been proposed.¹⁵ A third possibility is, therefore, that the Y chromosome long arm contains latent telomeric sequences which can be activated when a deletion event takes place within the fluorescent long arm, which would then allow replication to occur. Although the last proposal lacks experimental evidence, it might suggest either that repeated DNA sequences, specifically those represented by the 2000 copies of the pHY 2.1 probe present on the Y chromosome, are involved in the

function of the telomere, or that telomeric sequences are closely associated with these repeated DNA sequences. It is possible that the telomeric sequence in WSM 175 was located within the deleted chromatin and that this has made the Yq tip less stable with the subsequent formation of a ring XY bivalent. No stickiness which might have caused association between Yqh and other chromosomes in the somatic complement was apparently produced.

Finally, our results using the probe pHY 2.1 have confirmed the observations of Cooke *et al*⁸ showing that it hybridises predominantly to the tip of Yq 1.2. We differ, however, from Cooke *et al*⁸ who reported that this sequence was also present on the X chromosome. We did not detect this sequence on the X chromosome even after a 40 day exposure time, when the autosomes were clearly becoming specifically labelled. Because of the period of time left for grains to develop, the number of slides examined, and the number of metaphases scored, we must conclude that this sequence is absent from the X chromosome. Somatic cell hybrids containing only the human X chromosome have recently been probed with pHY 2.1 and no signal has been detected (H J Cooke, 1985, personal communication), supporting the above conclusion.

The authors are grateful to Dr A Carothers for carrying out the statistical analysis, to Dr A Busuttill (Pathology Department, Western General Hospital) for the histological report, and to Sandy Bruce of the MRC Photographic Department for the preparation of figures.

References

- 1 Handley AC, Goetz P, Hargreave TB, Joseph AM, Speed RM. On the nature and extent of XY pairing at meiotic prophase in man. *Cytogenet Cell Genet* 1984;**38**:241-7.
- 2 Sumner AT, Evans HJ, Buckland RA. New techniques for distinguishing between human chromosomes. *Nature* 1971;**232**: 31-2.
- 3 Sumner AT. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 1972;**75**:304-6.
- 4 Denton TE, Brooke WR, Howell WM. A technique for the simultaneous staining of both nucleolar organizer regions and kinetochore of human chromosomes with silver. *Stain Technol* 1977;**52**:311-3.
- 5 Evans EP, Breckon G, Ford CE. An air-drying method for meiotic preparations from mammalian testes. *Cytogenetics* 1964;**3**:289-94.
- 6 Schweizer D, Ambros P, Andrie M. Modification of DAPI banding on human chromosomes by prestaining with a DNA-binding oligopeptide antibiotic, distamycin A. *Exp Cell Res* 1978;**111**:327-32.
- 7 Howell WM, Black DA. Controlled silver staining of nucleolar organizer regions with a protective colloidal developer: a one-step method. *Experientia* 1980;**36**:1014-5.
- 8 Cooke HJ, Schmidtke J, Gosden JR. Characterisation of a human Y chromosome repeated sequence and related sequences in higher primates. *Chromosoma* 1982;**87**:491-502.

- ⁹ Joseph AM, Gosden JR, Chandley AC. Estimation of aneuploidy levels in human spermatozoa using chromosome specific probes and in situ hybridization. *Hum Genet* 1984;**66**: 234–8.
- ¹⁰ Mitchell AR, Gosden JR, Miller DA. p82H: a cloned sequence of the alphoid repeated DNA family found at the centromeres of all human chromosomes. *Chromosoma* 1985;**92**:369–77.
- ¹¹ Mitchell AR, Ambros P, McBeath S, Chandley AC. Molecular hybridisation to meiotic chromosomes in man reveals sequence arrangement on the No 9 chromosome and provides clues to the nature of 'parameres'. *Cytogenet Cell Genet* 1986;**41**:89–95.
- ¹² Solari AJ. Synaptonemal complexes and associated structures in microspread human spermatocytes. *Chromosoma* 1980;**81**:315–37.
- ¹³ Gosden JR, Gosden CM, Christie S, Cooke HJ, Morsman JM, Rodeck CH. The use of cloned Y chromosome specific DNA probes for fetal sex determination in first trimester prenatal diagnosis. *Hum Genet* 1984;**66**:347–51.
- ¹⁴ Cavalier-Smith T. Palindromic base sequences and replication of eukaryote chromosome ends. *Nature* 1974;**250**:467–70.
- ¹⁵ Dancis BM, Holmquist GP. Telomere replication and fusion in eukaryotes. *J Theor Biol* 1979;**78**:211–24.

Correspondence and requests for reprints to Dr A C Chandley, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh EH4 2XU.