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# The parental origin and mechanism of formation of three dicentric X chromosomes

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**Summary.** Cytogenetic and molecular analyses of three dicentric X chromosomes were performed in an attempt to identify the parental origin and mechanism of formation of the aberrant chromosomes. Results indicate that, in these three cases, the dicentric chromosomes were formed by chromatid breakage and reunion of sister chromatids at the breakpoint. In two cases the abnormal chromosomes were paternal in origin; in the third case the dicentric originated from the maternal X chromosome.

## Introduction

Various mechanisms have been suggested for the formation of dicentric X chromosomes. The first involves chromatid breakage with subsequent reunion of sister chromatids and splitting of the centromere. The resulting chromosome may be referred to as an isodicentric and consists of duplicated portions of a single X chromosome with symmetrical banding patterns on opposite sides of the breakpoint (Therman et al. 1974; Dewald et al. 1978). By the second mechanism, dicentric chromosomes arise by pairing and exchange of chromatids from homologous chromosomes (Therman et al. 1974; Therman et al. 1986). A chromosome formed in this manner is referred to as a homodicentric chromosome and may consist of equal or unequal portions of the homologous chromosomes (Ward et al. 1981). Dicentric chromosomes may also arise from crossover within an inversion loop (Therman et al. 1986).

The present report describes cytogenetic and molecular analyses of dicentric X chromosomes from three unrelated females. Southern blot analyses of DNA from the probands and available family members were performed to determine the parental origin and mechanism of formation of the abnormal chromosomes.

## Materials and methods

### *Case reports*

Case 1 (D.B.) was a 7-year-old white female evaluated because of minor stigmata of Turner syndrome (Stevenson et al. 1987). Case 2 (S.M.) was a 32-year-old white female evaluated because of premature menopause. Case 3 (A.R.) was a 17-year-old white female with primary amenorrhea and failure to develop secondary sexual characteristics.

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### *Preparations for cytogenetic analysis*

Peripheral blood lymphocytes were obtained for chromosome analysis on the three probands and available family members. Metaphase preparations were G-banded with the trypsin-Giemsa method of Seabright (1971). C-banding was performed after pretreatment with barium hydroxide and staining with Giemsa (Sumner 1972). High-resolution chromosome analysis was performed on prometaphase spreads prepared from amethopterin-synchronized lymphocytes (Yunis 1976). Chromosome preparations were R-banded by 5-bromodeoxyuridine (BrdU) incorporation followed by acridine orange staining (Pai and Thomas 1980).

### *Preparation of DNA and blot hybridization*

Genomic DNA samples were prepared from peripheral blood by the method of Bell et al. (1981) as modified by Cavenee et al. (1984). After the presence of high molecular weight material was confirmed, the genomic DNA was diluted to a concentration of 105 µg/ml. Genomic DNA was digested with various enzymes according to supplier specifications and fractionated by agarose gel electrophoresis (Schwartz et al. 1986). Southern transfer in 0.025 *M* $NaPO_4$ (pH 6.6), prehybridization, and hybridization were carried out as described previously (Schwartz et al. 1986). The DNA probes were radiolabeled by the method of Wyman and White (1980) to a specific activity of at least $2 \times 10^8$ cpm/mg.

### *DNA probes*

Three highly informative X chromosome probes were employed in this investigation. Probe St14 identified the locus DXS52 (Xq28) (Oberle et al. 1985), probe pDP230 identified the pseudoautosomal locus DXYS20 (Page et al. 1987), and probe S232 recognized the locus DXYS40 (Xp22) (Knowlton et al. 1986). A chromosome 4 probe, A1 (D4S12) (Gilliam et al. 1984), was used as an internal control for the densitometric studies.

*Densitometric analysis*

Dosage analysis of locus DXS52 was carried out using a Bio Rad model 620 video densitometer as described previously (Schwartz et al. 1987; Patterson et al. 1988).

## Results

*Cytogenetic analysis*

*Case 1* (D.B.). From the proband 100 metaphase spreads were examined: 50 cells had a 45,X constitution and 50 cells were 46,X,dic(X). C-banding revealed two blocks of centromeric heterochromatin on the abnormal X; one centromere appeared as a normal primary constriction and the other appeared to be inactive. High-resolution chromosome analysis revealed the breakpoint at Xp22 (Fig. 1); the proband's karyotype was designated 45,X/46,X,dic(X)(qter→p22::p22→qter). From R-banded preparations, a total of 25 cells were examined. The dicentric X was inactive in all cells studied. Parental karyotypes were normal with no evidence of mosaicism for X chromosome abnormalities. Chromosome analysis of three of the proband's sisters also revealed normal female karyotypes.

*Case 2* (S.M.). From S.M. 40 metaphase spreads were analyzed. In 32 cells there were 45 chromosomes with a single X chromosome; the remainder of cells had 46 chromosomes with 1 normal X and 1 dicentric X chromosome. Only one centromere was active on the dicentric chromosome, as confirmed by C-banding. High-resolution chromosome analysis identified the breakpoint on the dicentric as band p22.3 (Fig. 1). The proband's karyotype was designated 45,X/46,X, dic(X)(qter→p22.3::p22.3→qter). Parental karyotypes were normal with no evidence of mosaicism for X chromosome abnormalities.

*Case 3* (A.R.). From A.R. 50 prometaphase spreads were examined. In 7 cells there were 45 chromosomes with a single X chromosome; in the remaining 43 cells there were 46 chromosomes with a normal X and a dicentric X chromosome. The breakpoint on the dicentric was identified as q22 (Fig. 1), and the proband's karyotype was designated 45,X/46,XX,dic(X) (pter→q22::q22→pter). C-banding revealed one normal primary constriction and a second inactive centromere on the dicentric chromosome. The maternal karyotype appeared normal with no evidence of mosaicism for the dicentric chromosome; the proband's father was not available for chromosome studies.

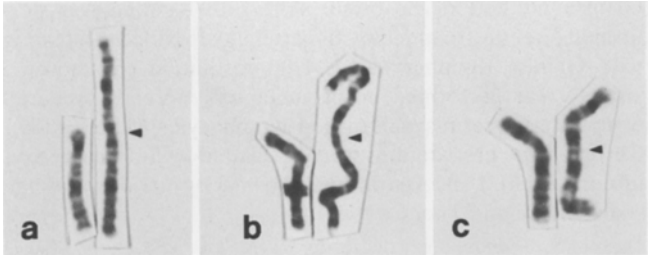


**Fig. 1a–c.** Representative pairs of G-banded X chromosomes from D.B. (**a**), S.M. (**b**), and A.R. (**c**). The dicentric X is on the right of each pair. The breakpoint on the dicentric X is indicated by an *arrowhead*

*DNA analysis*

*Case 1* (D.B.). The proband's father was not available for DNA analysis; therefore, his haplotypes were inferred by the study of two of the proband's sisters. Using the *Taq*I polymorphism detected by St14, the mother (P.B.) had alleles 6 and 7, the two sisters (B.B. and R.B.) had the haplotype 7,8 (Fig. 2a), and the proband's haplotype was 6,8; therefore, the paternal allele was presumed to be allele 8. Densitometric analysis revealed the two alleles in the proband to be of equal intensity.

Genomic DNA of the proband, her mother, and two sisters were further analyzed with two Xp probes localized to the region distal to the breakpoint of the dicentric X. Analysis with both pDP230 (Fig. 2b) and S232 (Fig. 2c) showed the proband had only bands present in the mother and possessed none of the presumptive paternal bands (those absent in the mother but present in the proband's sisters). These results

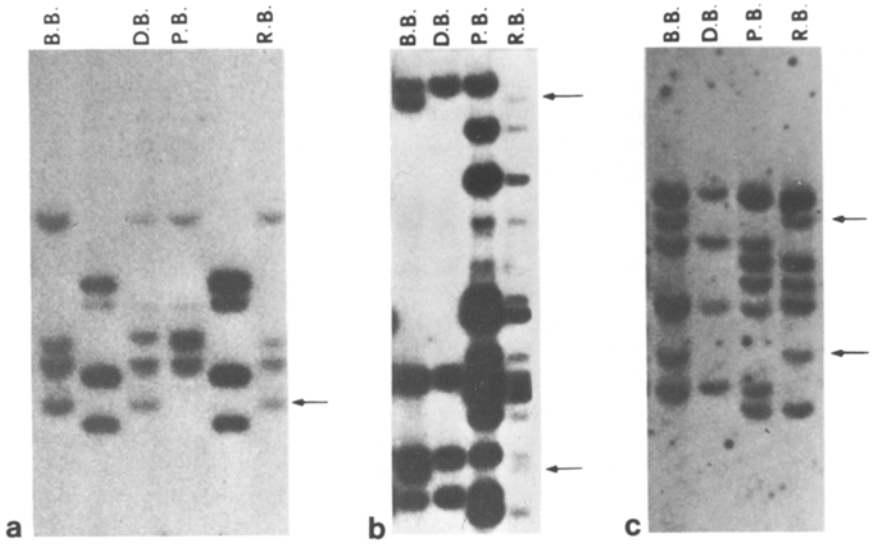


**Fig. 2a–c.** Southern blot analysis of D.B., her mother (P.B.), and two sisters (B.B. and R.B.) using polymorphic DNA probes localized to the X chromosome: **a** probe St14 on a *Taq*I blot, *lanes 2* and *5* are marker lanes containing fragments of pBR322; **b** probe pDP230 on a *Bgl*II blot; **c** probe S232 on a *Taq*I blot. *Arrows* indicate the location of paternally derived bands in B.B. and R.B.

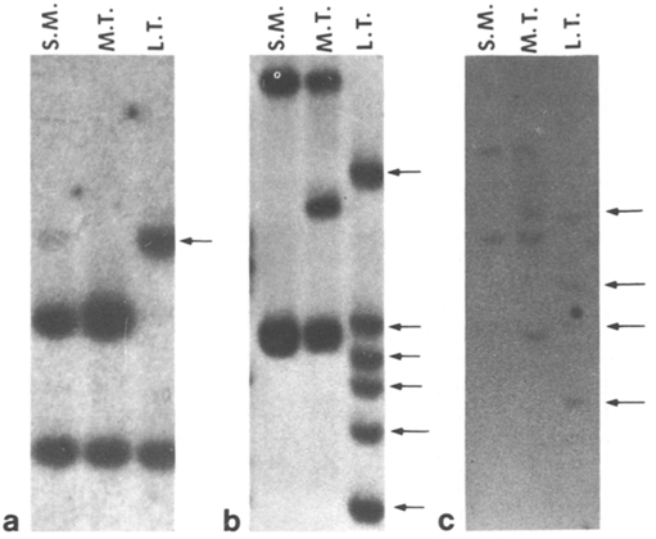


**Fig. 3a–c.** Southern blot analysis of *Taq*I genome digests of S.M., her mother (M.T.) and her father (L.T.): **a** probe St14, **b** probe pDP230, **c** probe S232. *Arrows* indicate location of paternal bands

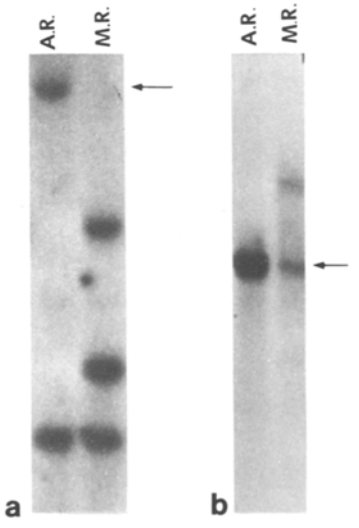


**Fig. 4a, b.** Southern blot analysis of *Taq*I genomic digests of A.R. and her mother (M.R.): **a** probe St14, **b** probe pDP230. *Arrows* indicate band of paternal origin

were confirmed using the *Taq*I and *Bgl*II polymorphisms of pDP230.

*Case 2* (S.M.). Using the St14 *Taq*I polymorphism, two alleles were detected in the proband (Fig. 3a). One allele, of maternal (M.T.) origin, was present at an intensity approaching that observed in a hemizygote. The second allele, of paternal (L.T.) origin, could be visualized upon longer exposure. Using the probe pDP230 (Fig. 3b) and S232 (Fig. 3c) the proband had only bands present in her mother.

*Case 3* (A.R.). The proband's father was not available for study; therefore, identity of the dicentric X was based on results obtained from DNA analyses of the proband and her mother (M.R.). Using the *Taq*I polymorphism of St14, the proband had a single allele that was not present in her mother (Fig. 4a). Studies with pDP230 revealed one very dark band in the proband and two lighter bands in her mother (Fig. 4b).

## Discussion

The purpose of the present study was to determine the mechanism of X dicentric formation and the parental origin of the aberrant chromosomes in three unrelated females. Dicentric X chromosomes may arise by isolocal reunion of sister chromatids, by chromatid breakage and reunion between homologs, or by crossing over within an inversion loop (Therman et al. 1986). Dicentric chromosomes may be maternal or paternal in origin. If a paternal origin of an X dicentric is established, the only possible mechanisms of formation are those uniting sister chromatids. A maternally derived X dicentric may derive from the reunion of sister chromatids (isodicentric) or from the joining of chromatids from homologous chromosomes (homodicentric).

In case 1 (D.B.) the mechanism of the dicentric's formation was determined by the use of the probe St14, which is specific for a locus in band Xq28 and detects a multiallelic system in *Taq*I digests. The proband has inherited alleles 6 (maternal) and 8 (paternal). Had the dicentric formed in a maternal germ line or meiotic cell by the joining of chromatids from X homologs, the proband would show three alleles: 6 and 7 from the mother and 8 from the father. The findings from St14 thus establish that the aberrant chromosome is not a homodicentric but an isodicentric. Densitometric data obtained from St14 films were consistent with the cytogenetic findings of the proband's mosaicism, with the dicentric X being present in about one-half of the cells. The duplication of a locus on the isodicentric appears in a direct gene dosage study as a normal disomic dosage. The parental origin of the dicentric was determined with pDP230, a probe that detects highly polymorphic sequences in the pseudoautosomal regions (Xp22.3 and Yp11.3) of the sex chromosomes (Page et al. 1987). As this locus is near the Xp telomere, it is deleted from the dicentric. The multiallelic systems revealed by pDP230 with *Msp*I, *Bgl*II, and *Taq*I show bands attributable to the paternal X chromosome in the proband's sisters and also delineate the segregation of the maternal X chromosomes. The proband lacks all of the paternal bands, having only bands present on the maternal X chromosome. The identification of the paternal origin of the dicentric was confirmed by the results from S232, a highly polymorphic probe of X chromosome origin specific for a locus just proximal to the pseudoautosomal region. Again, the proband had bands present in her mother and none found in her sisters that are presumed to be of paternal origin.

In case 2 (S.M.), the probe St14 was again used to determine the mechanism by which the dicentric formed. The intensity of the maternal allele was comparable to that seen in the hemizygous state, suggesting that the normal X, which was found in all cells examined cytogenetically, was of maternal origin. The reduced intensity of the paternal allele suggests that this allele is present on the dicentric X, which was found in only a small proportion of cells. The probes pDP230 and S232 were used to further investigate the parental origin of the dicentric X. Because these probes are localized to the region deleted in the dicentric, the lack of paternal alleles confirms that the dicentric chromosome arose from the paternal X.

In case 3 (A.R.), the breakpoint of the dicentric was at Xq22, leading to duplication of the entire short arm and proximal long arm of the X chromosome. Use of the probe St14 revealed a single allele in the proband, which was absent in the mother and presumed to be derived from the father. Since the

distal region of Xq was deleted in the dicentric, lack of the mother's allele indicated that the dicentric X was of maternal origin.

The probe DP230 was used to determine the mechanism by which the dicentric chromosome was formed. The mother was heterozygous at this locus and the proband had only one of the two maternal alleles, suggesting that the dicentric formed from a single maternal X chromosome. The intensity of the single band in the proband suggests that the paternal band on the normal X is the same size as the maternal band on the dicentric X.

Callen et al. (1987) used DNA markers to determine the paternal origin of a dicentric X chromosome, concluding that the dicentric arose by sister chromatid breakage and reunion and suggesting that this mechanism may also be significant for isochromosome formation. In the present study, DNA probes for the distal p and q arms were used to determine the method of formation and the parental origin of dicentric X chromosomes from three unrelated females. In each case, the aberrant chromosome was found to be an isodicentric derived from a single parental chromosome by the breakage of sister chromatids with subsequent reunion of chromatids at the breakpoint. In two of the individuals the isodicentric was derived from the paternal X chromosome whereas in the third it was of maternal origin. These studies demonstrate the power of DNA methods in the investigation of human dicentric chromosomes where the breakpoints, method of formation, and parental origin are uncertain.

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