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The Origin and Behavior of Two Isodicentric Bisatellited Chromosomes

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INTRODUCTION

Small bisatellited chromosomes are known to occur in normal and defective individuals [1–7] and are occasionally observed segregating within families [7–11]. C-banding has revealed that some of these chromosomes are dicentric [11–12]. A variety of hypotheses have been advanced to explain their origin, including centric fusion (Robertsonian translocation) and crossing over within an inversion loop of a heterozygous parent.

The present case suggests an alternative mechanism for the formation of an extra symmetrical dicentric chromosome during meiosis. Subsequent mitotic behavior explains the formation of other dicentrics and the evolution of a mosaic karyotype. The model can be generalized to encompass the formation of other extra dicentric isochromosomes and some instances of isochromosome X.

MATERIALS AND METHODS

Fibroblast and peripheral blood lymphocyte metaphases were cultured and prepared according to standardized procedures. The technique of sequential GT- to C-banding was modified from that of Lubs et al. [13]; the Q-banding methodology has been described by Lin and Uchida [14].

RESULTS

Case Presentation

The proband is a 7-year-old male. His birth weight was 2,891 g. Maternal age was 38 years at the birth of the proband. No teratogenic drugs or X-rays were prescribed during pregnancy, nor was there a history of infection. The physical appearance of the proband was normal except for alternating strabismus and a maxillary overbite. He was hyperactive and severely retarded; an EEG showed a diffuse disturbance of cerebral function. Laboratory studies, including urine screen for amino acids, reducing substances, and mucopolysaccharides, were normal. No unusual genotyping results were observed. Karyotypic examination is detailed below.

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The parents of the proband are first cousins of Palestinian descent. Of 12 siblings, two are in special education programs. Paternity was consistent by genotyping, and comparisons of autosomal variants and Y chromosome length.

Cytogenetic Findings

Examination of the proband's karyotype in 1974 demonstrated three cell lines: seven cells were apparently normal 46,XY; 13 cells showed an extra submetacentric chromosome of E group size; and five cells had a tiny metacentric chromosome in addition to the extra chromosome of E group size. When cultures were repeated for banding analysis 2 years later, the three cell lines could be more precisely characterized. One hundred four metaphase cells were counted; a normal male karotype was observed in 30 metaphases. Fifty-four cells had an extra acrocentric marker chromosome of E group size (M1), which had satellites on both the long and short arms. The remaining 20 cells had, in addition to the first marker, a second bisatellited chromosome (M2) which was the smallest chromosome in the karyotype. Both ends of M1 and M2 participated in satellite association.

There was no evidence of an inversion or other rearrangement involving the acrocentric chromosomes on examination of early metaphase chromosomes of the parents. Neither M1 or M2 was seen in 60 paternal or 60 maternal metaphase cells. Seven of the siblings, including the two in special education, had normal karyotypes.

Sequential G- to C-banding (fig. 1) demonstrated that both M1 and M2 were dicentric. Only one primary constriction was present in each marker. The C-band positive regions on M1 and M2 were similar in size to those on the no. 15 chromosomes of the patient and larger than the C-bands of the other acrocentrics. The marker chromosomes further resembled the no. 15 chromosomes in that their proximal C-bands extended into the short arm regions. Q-banding showed pale centromeres and satellites in both markers and similarly pale centromeres and satellites in both no. 15 chromosomes.

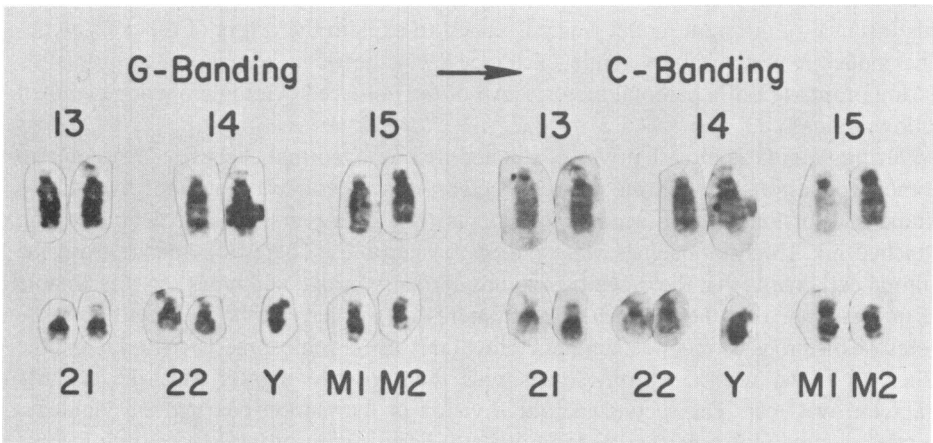

FIG. 1.—Partial karyotype of cell with both M1 and M2. Sequential G- to C-banding revealed that both markers were dicentric. Size and position of the C-band positive regions suggested that markers originated from chromosome 15 material.

DISCUSSION

Origin of the Marker (M1)

A mosaic karyotype in connection with the unusual extra marker chromosomes and normal parental karyotypes presented an opportunity to consider the evolution of the three cell lines. The presence of all three lines in multiple cultures suggested that each was also present in vivo. Because both markers had satellites and participated in satellite association, they were derived from an acrocentric chromosome or chromosomes. Occasional cells showed both ends of a marker involved in association. Sequential G- to C-banding (fig. 1) showed that M1 and M2 were dicentric and that all four regions of centromeric heterochromatin in the markers shared a common origin because of the similar size of all four bands. These large bands were also similar to the C-band region of the no. 15 chromosomes. Because of these similarities and because only chromosome 15 in either parent had similar centromere region morphology, we concluded that the markers originated from chromosome 15 material.

Examination of parental acrocentric chromosomes revealed no paracentric inversion, which we originally considered as a precursor to the dicentrics through the mechanism of crossing over within an inversion loop.

Another possible origin for the markers was that of centric fusion between the two no. 15 chromosomes, involving breakage in the proximal long arms of the homologs rather than immediately at the centromere. However, this was highly unlikely. If a centric fusion occurred in one parent, an independent nondisjunction of the normal no. 15 chromosomes in the other parent would have been required to net the proband two normal 15's; otherwise he would have had only one normal no. 15 chromosome. Also, there was no cytologic evidence that either parent carried M1 or M2, and the presence of two normal no. 15 chromosomes in the proband's karyotype excluded centric fusion in his own somatic cells as a possible origin of M1 and M2.

Since the usual explanations for the origin of bisatellited markers did not apply in this instance, a different model was postulated to explain the origin of this karyotype. The model requires only a single exchange event between homologous chromatids during prophase I of a parental meiosis. All of the observed cytologic phenomena then follow logically.

During parental meiosis, it was postulated that an abnormal exchange between two homologous (not sister) chromatids connected the two centromere regions by a single strand and left one or two acentric fragments (fig. 2). At first meiotic anaphase, the attached no. 15 chromosomes were pulled to one pole. (There is evidence from the mitotic cell counts that the dicentrics do not normally bridge and break; so it is tenable in meiosis too, that these attached centromeres do not have to be pulled to opposite poles.) This process left one daughter cell without any chromosome 15 material and the other with two no. 15 chromosomes plus the dicentric marker M1; the acentric fragment was lost. The second meiotic division of the marker-bearing cell yielded a normal gamete plus a marker-bearing gamete. Fertilization of the M1-bearing gamete resulted in the initial karyotype of 47,XY,+M1. Thus a single abnormal exchange event in meiosis prophase I produced a partial tetrasomy of chromosome 15.

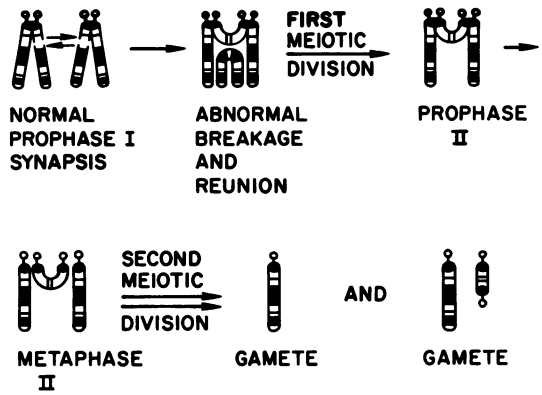

FIG. 2.—Theorized formation of marker M1 during meiosis. Breakage and abnormal reunion during first meiotic prophase resulted in formation of a dicentric isochromosome (M1) which was retained in the gamete; the resultant acentric fragment was lost.

The model allows for the inclusion of two normal and complete centromere regions in the dicentric. It does not explain the presence of two normal centromeres with only one primary constriction. It is doubtful that the physical constraint due to close proximity of the centromeres prevents the formation of two primary constrictions in the markers, because in certain dicentric X chromosomes where the C-band positive regions are widely separated [15–17], only one primary constriction is observed in most cells. While we do not understand the mechanism, there seems to be some intrachromosomal control over the behavior of the C-band positive regions.

Generalization of the Model

This model offers an alternative to the centric fusion or inversion model to explain the origin of other extra dicentric isochromosomes such as small extra bisatellited isochromosomes seen occasionally in normal subjects, and isochromosomes of 17p and 18p [18–21]. Few of these have been examined with C-banding, but one case presented by Balicek et al. [18] was karyotyped with R- and C-banding to demonstrate an isochromosome of 18p. Their C-banded karyotype illustrates an apparently dicentric isochromosome 18p, the C-band positive region being larger on the marker than on either of the normal no. 18 chromosomes. These findings are consistent with an origin by abnormal meiotic exchange between *homologous, nonsister* chromatids during parental meiosis.

The model can also explain the origin of dicentric X ischromosomes in subjects who have no normal 46,XX cell line [15, 22–23], as an alternative to the postzygotic intrachromosomal rearrangement concept introduced by German [24]. By the present model, an abnormal *sister* X-chromatid breakage and reunion in meiotic prophase I could lead to a gamate bearing a dicentric X-isochromosome (cf. [15]); fertilization with a normal X-bearing gamete would produce a zygote with the karyotype 46,X,i(Xq).

Behavior of M1 and Formation of M2

Since M1 was present in most of the cells, it appears to be relatively stable. If spindle fibers attached to the primary constriction region only, then M1 segregated normally to daughter cells to perpetuate the original karyotype. The same occurred even if both C-band positive regions were receptive to spindle fiber attachment, as long as fibers connected to one centriole were connected to one chromatid only. Consideration of spatial relationships between chromosomes and centrioles suggests that attachment of spindle fibers to only one chromatid best explains the normal segregation of M1.

The presence of other cell lines suggests that the C-band positive regions on M1 were occasionally receptive to spindle fiber attachment. If fibers at one centriole became attached to one chromatid at the primary constriction and to the other centromere region of the sister chromatid (fig. 3), then a bridge formed at anaphase. Breakage of the involved chromatid produced daughter cells with different karyotypes; both daughter cells had a fragment with "sticky ends," but only one daughter cell

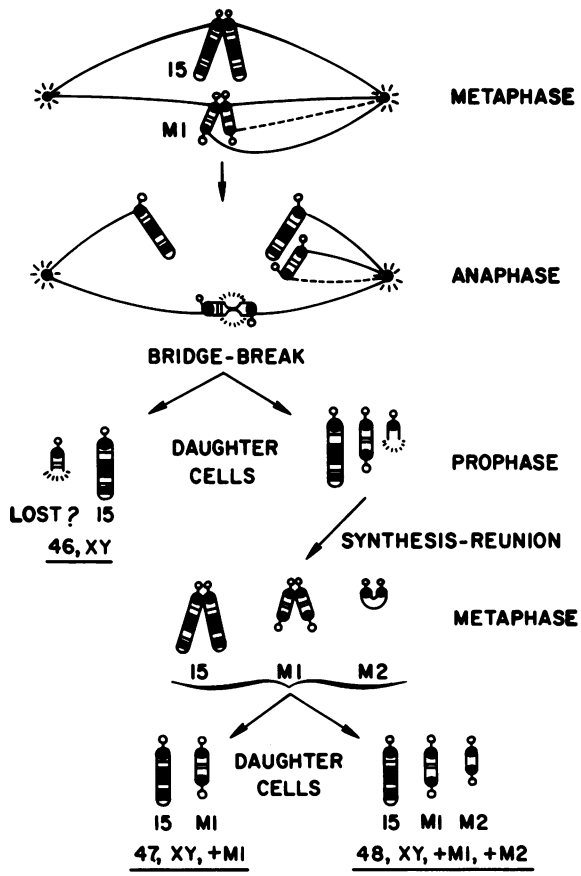

FIG. 3.—Abnormal mitosis producing the M2-bearing and the normal cell lines. See text for detailed description.

retained an intact marker M1. We believe that in the cell without M1, the sticky fragment was lost, producing the observed 46,XY cell line. In the other daughter cell, the fragment was not lost, but after the next synthetic phase, the "sticky ends" of the sister chromatids fused to form the marker M2. Daughter cells of this cell again differed in their karyotype, one daughter reverting to the original 47,XY,+M1 karyotype and the other having the observed 48,XY,+M1,+M2 karyotype.

The proband's karyotype can be described as 46,XY/47,XY,+idic(15)(pter→q15→pter)/48,XY,+i dic(15)(pter→q15→pter),+i dic(15)(pter→q12→pter). His phenotype and karyotype are not unlike those of subjects previously described as having partial trisomy 15 [5, 6, 25–31].

SUMMARY

Karyotyping revealed three cell lines in a boy with mental retardation and few other abnormalities. Thirty cells exhibited a normal karyotype, and 54 had an extra acrocentric chromosome of E group size with satellites on the long and short arms. The remaining 20 cells each had, in addition to the first marker (M1), a second tiny bisatellited chromosome (M2). C-banding demonstrated that both markers were dicentric. G-, C-, and Q-banding and satellite association data were consistent with the markers having originated from chromosome 15 material.

We propose that M1 was formed from a meiotic breakage and a chromatid fusion in the proximal long arms of an acrocentric pair. This would have produced a symmetrical isodicentric chromosome, plus one or two acentric fragments. M2 then could have resulted from a dicentric bridge-break-synthesis-reunion phenomenon. This model of abnormal meiotic exchange can be generalized to encompass the formation of other dicentric isochromosome cases of isochromosome X.

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Note added in proof: We are grateful to Drs. O. J. and Dorothy Miller and colleagues for applying their fluorescent anti-5-methylcytosine (anti-5MeC) technique to the present case. Anti-5MeC binds specifically to the short arm region of chromosome 15 after UV denaturation of the DNA. In our patient, anti-5MeC stains both ends of M1; M2 is still under examination. These preliminary results support our cytologic observations.

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