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Metaphase Chromosome Folds and X-Inactivation

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Abstract

To evaluate the suggested nonrandom folding of Xq13-q21 (center of Barr body condensation) of the inactivated X chromosomes, metaphases from nine subjects with or without X chromosome abnormalities (eight females and one male) were investigated with RBG-staining. A significant increase ($p < .05$) in the number of arm folds (Xq13-q21) of the late-replicating X chromosome, particularly in early to mid-metaphase, was observed in four of eight females. Therefore, the stage of chromosome contraction was an important factor with more folds observed at the centromeres and in longer chromosomes in early metaphase compared with mid- to late metaphase. X chromosome folds were present in cells of subjects treated with or without bromodeoxyuridine. While our study agrees with the relationship of Xq13-q21 fold with the X-inactivation center, the correlation of extended chromosomes and folding limits this method as a sole indicator of X-inactivation in routine mid-metaphase, but is useful in the analysis of early metaphase chromosomes.

Keywords

X chromosome folds; X-inactivation

INTRODUCTION

Flejter et al [1984] reported that bends in mitotic metaphase chromosomes were not distributed randomly throughout the karyotype and that nonrandom folding of the inactivated X chromosome occurs. The frequency of bends at centromeres correlated positively with the centromere index, while the frequency of bends in the noncentromeric regions correlated with the relative length of the chromosome arms. Furthermore, a bend between Xq13 and Xq21 or the center for Barr body condensation [Mattei et al, 1981] occurred more frequently than bends in any other chromosome region. Since this specific bend was confined to the inactivated X chromosome, Flejter et al [1984] proposed its use as a simple, accurate marker for identification of the inactive X chromosome.

Herein, we report our experience with metaphase chromosome folds and X-inactivation in studies of nine individuals with or without X chromosome abnormalities.

MATERIALS AND METHODS

Chromosome preparations were made in a routine fashion from cultures of peripheral blood lymphocytes. Four hours before harvesting, the cells were treated with 5-bromodeoxyuridine (BrdU). The RBG method [Dutrillaux and Viegas-Pequignot, 1981] was used for determination of X-inactivation. X chromosomes were analyzed for centromeric and arm folds (45 degrees or more from horizontal) using the criteria developed by Flejter et al [1984]. Approximately 75 cells from each of 9 subjects with or without an X chromosome abnormality (8 females and 1 male) were analyzed. The metaphase stage was recorded as early to mid- (approximately 500 bands), mid- (approximately 400 bands) and mid- to late (approximately 300 bands). Additional cells were also counted from routine culture preparations without BrdU treatment. Statistical analysis was by the chi-square test with one degree of freedom.

RESULTS

Examples of chromosome folds are shown in Figure 1 and the frequency of folds are presented in Table I. Two hundred seventy cells were scored as early to mid-metaphase; 310 cells were in mid-metaphase and 100 cells in mid- to late metaphase in the 9 subjects. Significantly more arm folds of the late X chromosome were observed in cases 3, 4, 5, and 8 ($p < .05$), with the majority of cells from these individuals in early to mid-metaphase. The highest frequency of arm folds (Xq13-q21) was 15/66 or 23% in the late X of case 8 (two Xq13-q21 regions present in the dup (Xq) chromosome), while only 1/66 or 2% of her early X chromosomes had an arm fold. Of the 15 Xq arm folds in case 8, 4 were in the Xq13-21 region and 11 in the dup (Xq13-28) region. There was no significant difference ($p > .05$) in the number of folds between the Xq13-q21 and dup (Xq13-28) regions. The lowest frequency of arm folds was 0% for the late and early X in cases 7 and 9, with the majority of cells in mid- to late metaphase. In the male subject (case 6), 4/95 or 4% of the X chromosomes possessed an arm fold. Therefore, a significant difference in the number of arm folds was noted only in cells that were scored in early to mid-metaphase and not in mid- to late metaphase. In the female subjects, centromeric folds were observed more frequently than arm folds (147 compared with 36, respectively).

One hundred twenty cells from four female subjects (with or without recognizable X chromosome abnormalities) were scored in routine chromosome preparation without BrdU treatment. Xq13-21 arm folds were also noted in the absence of BrdU treatment. For example, in case 8 [dup (Xq)], which has two Xq13-21 areas, 50 cells in early to mid-metaphase were analyzed in which the abnormal X was considered late replicating. Seventeen Xq 13-21 arm folds were observed in the abnormal X while no Xq13-21 arm fold was present in the normal X chromosome in cultures without BrdU compared with 14 Xq13-21 arm folds in the abnormal X and 1 Xq13-21 fold in the normal X in BrdU-treated cultures. Therefore, the frequency of Xq arm folds was not significantly different in BrdU-treated or nontreated cells.

DISCUSSION

We found a significant increase in folding of the late X chromosome in four of eight female subjects with or without an X chromosome abnormality, particularly in early to mid-metaphase. Our data agree with the results on nonrandom chromosome folding reported by Flejter et al [1984] in that bends were more common at the centromeres and occurred more frequently in longer chromosomes, such as the duplicated Xq (case 8) with two Xq13-21 regions. The stage of chromosome contraction appeared to be an important factor in that chromosomes with more folds were observed in early to mid-metaphase compared with mid- to late metaphase, in agreement with Flejter et al [1984]. Cells not treated with BrdU also showed the Xq arm fold of the late X more often than in the early X chromosome.

Conversely, an apparent lack of early X chromosome arm folds was noted. In 680 cells, only 1 early X possessed an arm fold while 35 late X chromosomes had arm folds. Fewer centromere folds were also recorded in the early X compared with the late X chromosome (60 and 87, respectively). The virtual absence of arm folds of the early X compared with the late X chromosome in BrdU-treated and nontreated cells is additional evidence that folding and inactivation are related.

As proposed by Flejter et al [1984], nuclear membrane attachment sites may play a role in chromosome folding. Chromosomes may bend at the primary constriction or other areas which may represent physically weak sites. A potentially weak area may be the site for Barr body condensation which has been localized to the region between Xq11.2 and Xq21.1 [Mattei et al, 1981]. The arm fold at Xq13-q21 may be a cytological manifestation of this condensation center.

While there appears to be a relationship of Xq13-21 bend with the X-inactivation center in early to mid-metaphase in case 3, 4, 5 and 8, our observations suggest that X chromosome folding may not be reliable as a sole indicator of X-inactivation in routine mid- to late metaphase. Because of better correlation between elongated chromosomes and folding, early metaphase preparations should be preferentially chosen in the analysis of X inactivation.

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Fig. 1.

Representative X chromosome pairs from a patient with a 46,X,dup(X)(q13-q28) karyotype stained with the RBG method. The abnormal X chromosome is late replicating and lightly stained. A) Single X chromosome fold of the Xq13-q21 region of the late-replicating X. B) Single X chromosome fold of the dup Xq13-q28 region. C) Folds in both the Xq13-q21 and dup Xq13-q28 regions.

TABLE I
Frequency of Folds at Centromere and at q13-q21 in Early- and Late-Replicating X Chromosomes in BrdU-Treated Cultures

Case No.	Karyotype	Metaphase stage*	Early X		Late X		P [†]
			Centromere folds	Xq13-q21 folds	Centromere folds	Xq13-q21 folds	
1	46,XX	Mid- to late	4	0	5	1	NS
2	46,XX	Mid-	8	0	13	3	NS
3	46,XX	Early to Mid-	10	0	19	7	<.01
4	46,XX,t(21q;?21q)	Mid-	6	0	16	4	<.05
5	46,X,fra(X)(q28)	Early to mid-	12	0	8	5	<.05
6	46,fra(X)(q28),Y	Mid-	14 centromeric folds; 4 q13-q21 folds				
7	46,X,del(X)(p22)	Mid- to late	6	0	4	0	NS
8	46,X,dup(X)(q13-q28)	Early to mid-	10	1	12	15	<.001
9	46,X,i(Xq)	Mid-	4	0	10	0	NS

NS = not significant

* Early to mid-metaphase stage—approximately 500 bands; mid-metaphase stage—approximately 400 bands; mid- to late-metaphase stage—approximately 300 bands.

[†] Early and late folding (q13-q21) based on two-tailed chi-square test.