0014-4754/94/050502-04$1.50 + 0.20

Increased incidence of unpartnered single chromatids in metaphase II oocytes in 39,X(XO) mice

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Received 26 August 1993; accepted 17 January 1994

Abstract. Since rare cases of sex chromosome anomalies such as XXX and XXY were observed in the offspring of our XO breeder mice, we performed a cytogenetic analysis of metaphase II oocytes of XO mice to determine whether any changes in chromosomal configurations occur. We found a significantly increased incidence of unpartnered single chromatids in metaphase II oocytes of XO mice. Such single chromatids may contribute to embryonic aneuploidy. In addition, the tendency of the X-chromosome to segregate non-randomly to the oocyte rather than to the polar body was confirmed.
Key words. XO mice; metaphase II oocyte; single chromatid; non-random segregation of the X-chromosome.

Unlike the corresponding situation in humans, female mice with an XO sex chromosomal complement are morphologically normal, fertile and breed with only a slight reproductive disadvantage[1]. During the course of cytogenetic screening for breeding of our XO mouse colony, we have encountered rare cases of sex chromosome anomalies such as XXX and XXY in the offspring of XO breeder mice[2–4]. X-trisomy in the mouse, in particular, had not been reported previously in the literature. Therefore, we speculated that meiosis at the stage of oocyte production is not regulated properly in XO individuals. The present study was designed to determine whether any changes in chromosomal configurations occur in metaphase II oocytes of XO mice.

In addition, we intended in this study to investigate further the still unsettled question of the segregation ratio of X-bearing oocytes to nullo-X oocytes at oogenesis in XO mice. While some investigators[5,6] found preferential elimination of nullo-X oocytes at meiosis I, other groups of investigators[7,8] have not observed this phenomenon.

Materials and methods

All mice used in this study were obtained from our XO mouse colony, which was established 7–8 generations ago from XO offspring of JcI/ICR females exposed to X-rays after copulation[2]. The animals were maintained at a room temperature of 23 ± 2 °C at 50 ± 10% RH with a 12-h light/dark cycle (lighting 08:00–20:00).

Since artificial induction of ovulation is suspected to be a possible cause of chromosome anomalies[9,10], oocytes were obtained after spontaneous ovulation in this study. To obtain timed oocytes, virgin XO and XX mice (JcI/ICR: 3–6 months old) were mated to male mice of the same colony between 09:00–10:00 h (early morning short period mating). At the end of the mating period, the females were checked for the presence of a vaginal plug. Plug-positive females were immediately killed by cervical dislocation, and oocytes were collected from the ampullae of the oviduct. Chromosome preparations of metaphase II of meiosis were made by the air-drying method of Tarkowski[11]. Slides were aged at 37 °C for about 10 days and C-banded according to the procedure of Sumner[12], which facilitated more accurate counting of the chromosomes.

Since our preliminary observation had indicated a fairly high incidence of unpartnered 'single chromatids' (half of the whole chromosome which prematurely split into two chromatids) in metaphase sets in XO mice, we first determined the incidence of such single chromatids in each metaphase II oocyte set. Then, the total number of chromosomes was determined. For this, oocytes with insufficient chromosome spreading or too much spreading, those with less than 18 chromosomes, and those with poor C-band staining were excluded from the analysis. To avoid bias during the observation, each slide was examined blind.

Results

Table 1 shows the incidence of single chromatids in metaphase II oocytes from XO and XX females. A highly significant increase in single chromatids was observed in oocytes from XO mice. Table 2 presents the distribution of chromosome counts with single chromatids. Most of these oocytes had either one or two single chromatids with either 18 or 19 chromosomes. When two single chromatids were present, their length seemed to be almost the same, although they lay far apart from each other. In these oocytes, the centromere regions of other chromosomes remained joined. Since no G-banding technique was used in this study, it was

Table 1. Incidence of single chromatids in MII oocytes from 39,X(XO) and 40,XX mice

	Litters	No. of oocytes obtained	No. of oocytes analyzed	No. of oocytes with single chromatids
XO	12	219 (18.2)[a]	141 (64.4)[b]	41 (29.1)[c]*
XX	13	205 (15.8)[a]	136 (66.3)[b]	8 (5.9)[c]

[a]Average no. of oocytes per litter; [b]percent of analyzed oocytes; [c]percent of oocytes with single chromatids; *$p < 0.001$ when compared with XX group (by the chi-square test).

Table 2. Distribution of chromosome counts with single chromatids

	Oocytes with single chromatids	Chromosome counts with single chromatid (C)									
		13 +10C	13 +14C	15 +10C	17 +2C	18 +1C	18 +2C	18 +4C	19 +1C	19 +2C	20 +2C
XO	41	1 (2.4)*	0	2 (4.9)*	1 (2.4)*	2 (4.9)*	14 (34.1)*	1 (2.4)*	13 (31.7)*	6 (14.6)*	1 (2.4)*
XX	8	0	1 (12.5)*	0	0	0	2 (25.0)*	0	2 (25.0)*	3 (37.5)*	0

*Percent of oocytes with respective numbers of single chromatids.

impossible to identify the chromosomes involved in the respective oocytes. However, judging from the relative sizes of the chromosome in question, it does not seem that any specific chromosomes are prone to this phenomenon.

Chromosome counts of metaphase II oocytes of the XO and XX groups are compared in table 3. Two single chromatids of a similar size were presumed to derive from one chromosome. A higher incidence of hypoploid ($n = 19, 18$) oocytes from XO mice naturally comes from the presence of nullo-X oocytes derived from a disjunctional event at meiosis I of 39,X primary oocytes. Hypoploid oocytes observed in XX mice may be either technical artifacts or be due to segregational events such as nondisjunction and anaphase lag. If we assume that, in XO mice, the segregation of the X chromosome to the oocyte or to the polar body was random, and that hypoploidy occurred at the same incidence as in XX mice, the expected distribution of oocytes with chromosome counts of 18, 19 and 20 in the XO mice would be 21, 65 and 54 respectively. However, our observed distribution (8, 51, and 81, when chromo-

Table 3. Chromosome counts of MII oocytes from 39,X(XO) and 40,XX mice

	No. of oocytes analyzed	Chromosome counts			
		18	19[a]	20[b]	21
XO	141	8 (5.7)[c]	51 (36.2)[c]	81 (57.4)[c]	1 (0.7)[c]
XX	136	9 (6.6)[c]	22 (16.2)[c]	105 (77.2)[c]	0 (0)[c]

[a]Including oocytes with 18 chromosomes + 1 single chromatid; [b]including oocytes with 19 chromosomes + 1 single chromatid; [c]percent of oocytes with respective chromosome numbers.

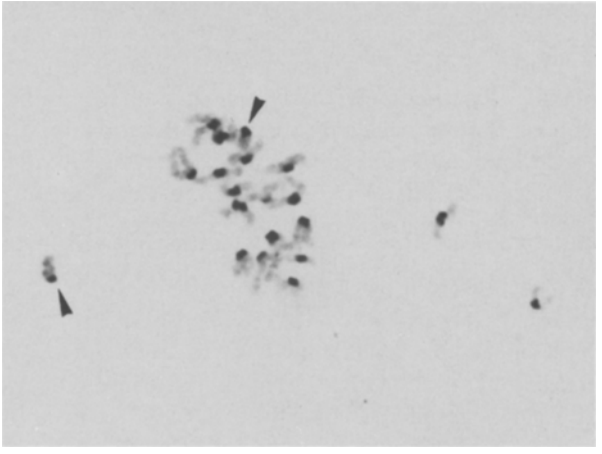

Figure 1. C-banded metaphase II oocyte from an XO mouse. Two unpartnered single chromatids (arrow) are present.

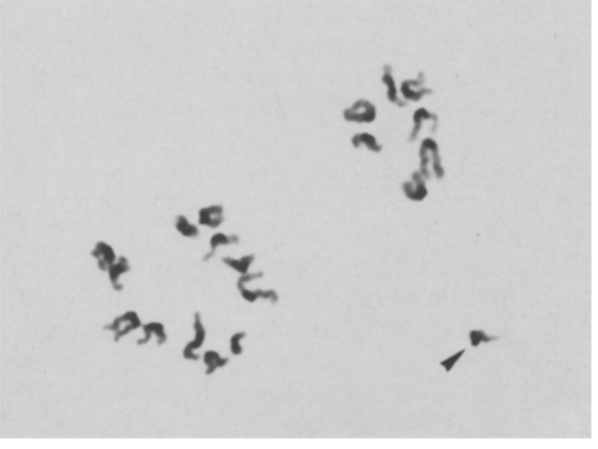

Figure 2. C-banded metaphase II oocyte from an XO mouse. One unpartnered single chromatid (arrow) is present.

some counts of 18.5 and 19.5 were taken as 19 and 20) differed significantly from this ($p < 0.001$, by the chi-square test), showing a definite tendency toward non-random segregation of the X-chromosome to the oocyte.

Discussion

The present study is the first to show that metaphase II oocytes in XO mice have a highly increased incidence of single chromatids. Previously, the occurrence of single chromatids was observed in mouse metaphase II oocytes under various circumstances[9,13–20]. Some investigators[9,13,14] speculated that single chromatids may result from 'presegregation' of the univalent chromosome at anaphase I, while others[17–20] proposed that 'premature' centromere separation or 'early segregation' at prometephase II is responsible. However, presegregation requires both univalent formation and the separation to the sister chromatids of such univalents. The frequency of single chromatids in our study was too high to suppose that two such events are occurring simultaneously at meiosis I. Moreover, Polani and Jagiello[13] suggested that univalents do not always result in presegregation. We assume that 'premature' centromere separation or 'early segregation' at prometephase II may be a more likely explanation for the observation in the present study. MII oocytes with only one single chromatid may result from chromatid loss during slide preparation. Besides, as shown in table 2, multiple single chromatids occur in more than half of the oocytes which have single chromatids. In this connection, we occasionally observed two single chromatids of a similar size still lying near to each other. We speculate that, in XO mice, the high incidence of single chromatids resulted from either increased centromere fragility or alteration of the meiotic spindle. Interestingly, Rodman[21] has demonstrated an increased rate of disjoined chromatids in postovulatory aged metaphase II oocytes.

A connection between single chromatids and subsequent embryonic aneuploidy has been suspected[9,13,15,19]. If two separated chromatids (19 + 2C) come from one X-chromosome and they did not segregate evenly at meiosis II, one of the derived oocytes would eventually produce individuals with XXX or XXY sex chromosome complements when fertilized by spermatozoa. This would explain the rare cases of XXX and XXY sex chromosome anomalies in our breeding colony. (So far, we have observed four XXX individuals in about 900 female offspring born to our XO breeder mice.)

Earlier Lyon and Hawker[22] predicted that chromosomal nondisjunction may occur at a high frequency in XO females since their estrous cycle may become irregular toward the end of their reproductive period, and they may show hormonal imbalance. However, old XO mice were not used in the present study. The numbers of oocytes recovered did not differ between the groups, and no increased incidence of degenerated oocytes was detected in the XO mouse group. Therefore, our present finding may not be related to precocious ovarian aging.

The reported XO/XX ratio in female offspring born to XO mice falls mostly between 1:2–1:3 among different breeding colonies[1,2,8,23,24]. Preferential segregation of the X-chromosome to the oocyte rather than to polar body I is one possible explanation for this phenomenon[25]. By analyzing metaphase II oocytes from XO mice, Evans[7] and Brook[8] showed that segregation of the X-chromosome to the oocyte or to the polar body is random, the ratio of 19 to 20 chromosomes being approximately 1:1. However, Kaufman[5] and Luthardt[6] showed a ratio of 1:2, suggesting preferential segregation to the polar body I of nullo-X oocytes. If we follow Brook's correction model for calculation of the adjusted ratio[8], our ratio would become 1:2.4 (95% Cl: 1.70–3.66), when the chromosome counts of 18.5 and 19.5 were taken as 19 and 20, and oocytes with 18 and 21 chromosomes were excluded from the calculation. However, the finding of high incidence of single chromatids in the XO mouse group in the present study indicates that the segregational events in XO mice may differ from those in XX mice, and it is unlikely that the chromosome (chromatid) loss occurs in the same manner as in XX mice. Especially, single chromatids may be more likely to be lost from the metaphase plate. Therefore, we should be cautious in interpreting our calculated ratio itself, although we may be fairly certain that the X-chromosome is preferentially segregated in the first meiosis of XO mice. Incidentally, in neither of the previous reports on X-chromosome segregation analysis description of 'single chromatids' could be found. The topic of 'single chromatids' must have been too new at the time of publication.

In the offspring from 47 XXX human females, the incidence of sex chromosomal aneuploidy is substantially less than expected[26]. One possible explanation for this may be that during meiosis the chromosome sets which have XX are more likely to be segregated to the polar body[26,27]. There may be some meiotic mechanism to maintain euploid gametes in the process of oogenesis in aneuploid females.

We conclude, first, that in XO mice single chromatids appear at a high frequency in metaphase II oocytes, and, second, that the X-chromosome tends to remain in the oocyte rather than being segregated to the polar body.

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