X-chromosome inactivation and ovarian age during the reproductive years

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Objective: To explore whether skewed X-chromosome inactivation (XCI) is related to indicators of ovarian age.
Design: The XCI skewing percent and indicators of ovarian age were measured in women with recent pregnancy losses and women with recent livebirths. All analyses adjust for chronologic age and pregnancy outcome.
Setting: Hospital in eastern central New York.
Patient(s): One hundred thirty-six women with informative XCI assays: 83 with index pregnancy losses and 53 with livebirths.
Intervention(s): None.
Main Outcome Measure(s): The primary indicators of ovarian age were antral follicle count, levels of FSH and inhibin B. A secondary indicator was level of estradiol (E_2).
Result(s): The XCI skewing percent, defined either continuously or categorically (≥90%), was unrelated to the indicators of ovarian age. The sample was large enough to rule out as unlikely a modest decline in antral follicle count or a modest increase in FSH in relation to skewed XCI.
Conclusion(s): X-chromosome anomalies are associated with skewed XCI and with premature ovarian failure. Our data raise the possibility that X-chromosome anomalies may not be an important influence on ovarian aging in menstruating women. (Fertil Steril® 2006;85:1488–95. ©2006 by American Society for Reproductive Medicine.)

Key Words: X-chromosome inactivation, ovarian age, antral follicle, premature ovarian failure, epidemiology, FSH, inhibin B

In women, one of the two X chromosomes in each cell is inactive, although some genes—perhaps about 15%—escape inactivation (1). X-chromosome inactivation (XCI) occurs at an early stage of embryonic development, probably when there are 4–16 cells (2–4), and is propagated in all the cell's descendants. In most females, which X chromosome becomes inactivated (i.e., the maternal or paternal X) is a random process. Thus, on average, 50% of active X chromosomes are maternal in origin, and 50% are paternal. Only a small proportion of women will demonstrate an extreme XCI skewing percent (usually defined as ≥90% of cells with the same active X chromosome) by chance alone.

Skewed inactivation can also arise from preferential inactivation of one X chromosome (e.g., if one X is structurally abnormal or has mutations that interfere with cell survival or growth (reviewed by Belmont (5)). Preferential inactivation can occur at the time of the initial inactivation or secondarily, over time, as cells replicate, due to selection of cells inactivating either the normal or abnormal X chromosome. Thus, the presence of extreme skewing may identify instances where an X chromosome is abnormal, although the frequency of X-chromosome anomalies among women with extreme skewing is unknown. Extreme skewing may also arise over time due to random drift. A recent longitudinal study suggests that selection for cells with a particular X chromosome active is probably not a continuous process: the XCI skewing percent did not change with age (over an average of 16.5 years) until after age 60 (6).

X-chromosome abnormalities are also an uncommon but established cause of primary amenorrhea and of premature ovarian failure (POF) (reviewed by Laml et al. (7) and by Simpson and Rajkovic (8)). For example, monosomy X (complete or partial), deletions on the X chromosome, and fragile X premutations are associated with primary amenorrhea or with POF. Premature ovarian failure, defined as cessation of ovarian function after puberty and before age 40 (9), may reflect either a small pool of primordial follicles or accelerated atresia. It is unclear whether POF constitutes the tail of a continuum in the distribution of age at menopause or a discrete disorder with a different pathogenesis.

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Only one case-control study has tested the hypothesis that extreme XCI skewing is associated with POF. In a Japanese sample, skewing ≥90% occurred in 5 of 24 women with POF and none of 29 controls of similar age. Because the analysis excluded women with identified X-chromosome anomalies and low-level monosomy X mosaicism, the authors hypothesize that cryptic anomalies or gene mutations on the X chromosome underlie both extreme skewing and ovarian failure. If so, we might expect X-chromosome anomalies to be associated with indicators of older ovarian age even among menstruating women.

This article tests the hypothesis indirectly. We draw on already collected data to explore whether or not extreme skewing is associated with ovarian age older than that expected, given chronologic age. The primary indicators of ovarian age are antral follicle count and levels of follicle-stimulating hormone (FSH) and inhibin B; we also provide data on estradiol (E_2).

MATERIALS AND METHODS

Participants

Selection criteria and the protocol for this study are described in detail elsewhere (10). Our Institutional Review Board approved the study. The sample derives from a study designed to test the hypothesis that the association between advancing chronologic age and trisomic pregnancy reflects an association between the size of the oocyte pool and trisomy, with risk higher in women with fewer oocytes. The data did not support the hypothesis (10).

The Protocol

Briefly, from September 1998 to April 2001, we identified women age 18 or older with singleton prefetal losses (developmental age less than 9 weeks) whose products of conception were submitted to the pathology department of a hospital in eastern central New York State. We asked for permission to karyotype the abortus. If a woman's loss was successfully karyotyped, we asked her to complete a short telephone interview to determine her eligibility. Eligible women who consented to the protocol completed a more extensive telephone interview and made two visits to the study hospital during the first week of their second or later menstrual cycle: the first on day 1–4 for a blood draw and the second on day 5–7 for transvaginal sonography. A portion of the blood sample was saved for analysis of X aneuploidy. During the study, we discovered that some samples had been processed incorrectly; we asked those women for a second sample for genetic studies.

To obtain valid ovarian age measures, we required the following: no pituitary disorder or hormonal disorder related to ovarian function, no oophorectomy, no hormonal medication, no pregnancy at the time of ultrasound, no breastfeeding or breastfeeding no more than once per day during the menstrual cycle preceding the study assessments. We required that any diagnosis be current, that the report of the diagnostic work-up be informative, and that the clinical symptoms and treatment be consistent with the diagnosis.

Women with Spontaneous Abortions

Of the 244 women with karyotyped losses, 127 (52%) completed the protocol (Table 1). The principal reasons for not completing the protocol were refusal (23%) and ineligibility (25%), primarily due to use of hormonal contraceptives or pregnancy soon after the index loss.

Women with Livebirths

For each woman with a trisomic loss who completed the study, we selected an age-matched control with a chromosomally and anatomically normal livebirth ≥1,800 g, no pregnancy loss since the index pregnancy, and no known trisomic pregnancy. They were selected from the hospital delivery log of women who delivered during the 7–13 months preceding the date of selection. Livebirth controls were matched to trisomy cases for projected age (±6 months) at the sonography visit. If a selected control was ineligible for the study or refused to participate, we replaced her. The protocol for women with livebirths was identical to the protocol for women with prefetal losses.

In total, we selected 219 women with livebirths, 65 of whom (30%) completed the protocol (Table 1). The principal reasons for not completing the protocol were refusal (31%) and ineligibility (37%), primarily due to use of hormonal contraceptives or breastfeeding.

Analytic Sample

To maintain independence of observations, analyses exclude repeat study entrances of four women. They also exclude 52 women for whom we could not determine the XCI skewing percent either because [1] the blood sample was not saved for DNA analysis or produced insufficient DNA (n = 44), or [2] the XCI analysis was uninformative because the two X chromosomes were homozygous at the androgen receptor (*AR*) locus (n = 8).

Characteristics of the Sample

Among the 136 women with informative XCI assays, 83 had an index pregnancy ending in spontaneous abortion (42 trisomies, 17 other chromosomally abnormal, 13 chromosomally normal male, 11 unknown karyotype) and 53 an index pregnancy ending in livebirth. Average age at ultrasound was 34 years (range 22–48). The majority were white (93%) and had completed high school (97%). Ninety-six percent completed the blood and sonography protocols after the second or third menstrual period following the index loss or, for women with livebirths, the introductory letter.