Skewed X-chromosome inactivation and shorter telomeres associate with idiopathic premature ovarian insufficiency

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Objective: To analyze whether telomere length, X-chromosome inactivation (XCI), and androgen receptor (*AR*) GAG polymorphism are related to idiopathic premature ovarian insufficiency (POI).
Design: Case–control study.
Setting: University hospital.
Patient(s): A total of 121 women, including 46 nonsyndromic POI and 75 controls.
Intervention(s): None.
Main Outcome Measure(s): Age, weight, height, body mass index (BMI), systolic and diastolic arterial pressure, E_2, androstenedione, T, and C-reactive protein were assessed. Telomere length was estimated by quantitative real-time polymerase chain reaction, XCI was measured using the Human Androgen Receptor and X-linked retinitis pigmentosa 2 (*RP2*) methylation assays. *AR* and *FMR1* polymorphism was assessed by quantitative fluorescent polymerase chain reaction and sequencing.
Result(s): Premature ovarian insufficiency women had a higher mean age, weighed less, and exhibited lower C-reactive protein, E_2, and androstenedione levels. The *AR* polymorphism did not differ between the groups. Four patients had premutation (55–200 CGG repeats), and none displayed a full mutation in the *FMR1* gene. However, patients with POI showed shorter telomere length and higher frequency of skewed XCI. Extreme skewing ($\geq$90%) was observed in 15% of women with POI, and shorter telomeres correlated with XCI skewing in both groups.
Conclusion(s): Skewed XCI and shortened telomere length were associated with idiopathic POI, despite no alterations in the *AR* and *FMR1* genes. Additionally, there is a tendency for women with short telomeres to exhibit skewed XCI. (Fertil Steril® 2018;110:476–85. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Anovulation, epigenetic mechanisms, DNA methylation, trinucleotide repeats, genomic instability

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Premature ovarian insufficiency (POI), also known as premature ovarian failure, is a common cause of female infertility, affecting approximately 1% of women of reproductive age. Premature ovarian insufficiency is defined as the premature cessation of ovarian function before 40 years of age. Primary or secondary amenorrhea, elevated serum gonadotropins, such as FSH levels >40 IU/L, and hypoestrogenism, also referred to as hypergonadotropic hypogonadism, are among the main characteristics of the disease (1, 2). In most cases the etiology of spontaneous POI is unknown, but there is evidence suggesting a genetic component in idiopathic ovarian insufficiency (2). X-chromosome-linked abnormalities have been reported in POI (3), including, for example, the expansion of trinucleotide repeats in genes such as fragile X mental retardation 1 (*FMR1*) (4).

The androgen receptor (*AR*) gene, located in the Xq12 region, contains a repetitive (CAG)n polymorphism that is associated with ovarian function and folliculogenesis (5). The expression of these repetitive sequences remains under epigenetic control, and the allele containing the expansion is often silenced through hypermethylation of the repeat and its regulatory regions (6, 7). In the inactive X chromosome, the CAG repeat in the exon 1 region of the *AR* gene is methylated and leads to the silencing of this gene. The methylation pattern of *AR* is the primary marker used to identify X-chromosome inactivation (XCI). Several epigenetic modifications are responsible for the inactivation of one of the two X chromosomes (X_i), resulting in the silencing of the majority of genes on X_i. Deoxyribonucleic acid methylation also plays an important role in the maintenance of this inactive state. X-chromosome inactivation is a random process, with an average 50:50 ratio of cells expressing either the maternal or paternal chromosome (8, 9). Nonrandom (or skewed) XCI is rare and related to X-linked disorders (10).

Mechanistically, the exhaustion of the ovarian reserve and subsequent reduction in female fertility may be due to abnormalities acquired over successive cell cycles, leading to oocyte senescence, which is a defining feature of POI (11). Decreased fertility in women with POI may be attributable to the depletion of the primordial follicular pool, resulting in a premature reproductive aging phenotype (3). The loss of the cellular proliferative capacity is a natural consequence of aging and is accompanied by genomic instability and telomere attrition. Telomere erosion is associated with reproductive senescence in women (12), which typically occurs after 40 years of age. This is mainly due to a reduction in the size and quality of the oocyte/follicle pool and the consequent loss of fertility (12–15). Indeed, the meiotic dysfunction that follows telomere loss reduces oocyte developmental competence and quality (14). Liu et al. (13) demonstrated that female germ cells do not undergo apoptosis after telomere shortening but are arrested in early meiosis and become aneuploid, suggesting that telomere shortening is associated with poor oocyte quality.

The intricate network of genomic interactions controlling the gonadal development provides the basis for understanding a complex disease such as POI (16). It is possible that several X-linked epigenetic markers, such as nonrandom X-inactivation or cryptic mosaicism (45,X/46,XX), *AR* (CAG)n expansion, or telomere dysfunction, may be related to oocyte senescence and loss of fertility in women with POI. In this context we sought to evaluate the association between telomere length, *AR* repetitive CAG polymorphism, skewed XCI, and the development of idiopathic ovarian insufficiency.

MATERIALS AND METHODS

Participants

The protocols were approved by the research ethics committee of the University Hospital (institutional review board) of Ribeirao Preto Medical School, University of São Paulo (protocol number 13305/2012) and ratified by the other participating institutions. All participants provided written, informed consent.

This was a prospective, case–control study recruiting consecutive patients from 2012 to 2016. Participants with POI (n = 46) were recruited at three academic medical centers in southeastern Brazil, including the Human Reproduction Division of the Department of Gynecology and Obstetrics of the Ribeirao Preto Medical School, University of São Paulo (FMRP-USP) (n = 23) and the Gynecology and Obstetrics Department of the University of Campinas (n = 13) and Federal University of Minas Gerais (n = 10).

Women aged 18–41 years, regardless of race, social status, or parity, were eligible. The idiopathic POI group (n = 46) included women with a history of amenorrhea and two serum FSH results >40 IU/L obtained before 40 years of age. At the time of the study the absolute majority of POI patients were receiving hormone therapy with oral or percutaneous E_2 and oral progestogen, and none was on androgen therapy. A 46,XX karyotype was required for women younger than 30 years. The control group (n = 73) was recruited at the University Hospital, FMRP-USP and included women with regular menstrual cycles of 24–38 days and a typical duration of 3–7 days, with no history of anovulation and FSH level <10 IU/L. The exclusion criteria were tobacco smoking, pregnancy or lactation, and a history of any other endocrine disorder. Syndromic POI cases (such as Turner's and Fragile X syndrome) and patients with autoimmune disorders, a history of radio- or chemotherapy, ovarian surgery, and chromosome abnormalities were excluded from the study.

Biochemical Measurements

Clinical and anthropometric characteristics were assessed during the study, including age, weight, height, body mass index (BMI), and systolic and diastolic blood pressure. The serum concentrations of E_2, luteinizing hormone (LH), follicle stimulating hormone (FSH), and C-reactive protein (CRP) were measured using chemiluminescence assays (IMMULITE 2000 Immunoassay System, Siemens Healthcare Diagnostics), whereas serum testosterone (T) and androstenedione (A) concentrations were measured using radioimmunoassays (Immulite 1000, Siemens Healthcare Diagnostics).