Premenopausal Amenorrhea:
What’s in a Number?

Robert D. Nerenz,1 Emily S. Jungheim,2 and Christina M. Lockwood1*

CASE DESCRIPTION

A 33-year-old nulligravid woman presented to her gynecologist after experiencing 5 months of secondary amenorrhea following discontinuation of oral contraceptives. She had undergone normal puberty and menarche with no significant past medical or surgical history. Her family history was unremarkable other than a brother with developmental delay. On exam she was 165 cm tall and weighed 67 kg, with a body mass index of 24.6 kg/m² (reference interval, 18.5–24.9 kg/m²). She did not display any evidence of hyperandrogenism (hirsutism or acne), and her pelvic exam was normal. A urine pregnancy test was negative. Initial progestin challenge testing (daily oral administration of 10 mg medroxyprogesterone acetate for 7 days) was negative (no bleeding), indicating that the patient was either hypoestrogenic or that she had uterine outflow abnormality (e.g., uterine scarring) (1). (Progestin challenge testing provides an indirect assessment of serum estradiol concentrations. Due to false positives and negatives associated with progestin challenge testing, as well as the development of assays directly measuring serum estradiol, progestin challenge tests are not routinely used.) A uterine outflow abnormality was deemed to be unlikely given that the patient had normal menses on oral contraceptive pills and she had no history of uterine manipulation or surgery. Her serum prolactin was 8.3 ng/mL (reference interval, 0.2–24.9 ng/mL), her total testosterone (measured by chemiluminescent immunoassay) was 14 ng/dL (reference interval, 14–76 ng/dL), and her free testosterone (measured by liquid chromatography–tandem mass spectrometry) was 0.2 pg/mL (reference interval, 1.3–9.2 pg/mL). Her serum thyroid-stimulating hormone (TSH) was 3.03 μIU/mL (reference interval, 0.36–4.20 μIU/mL) and her follicle-stimulating hormone (FSH) was 57.3 mIU/mL (reference intervals: follicular phase, 2.5–10.2 mIU/mL; midcycle, 3.4–33.4 mIU/mL; luteal phase, 1.5–9.1 mIU/mL; postmenopausal, 23.0–116.3 mIU/mL), confirming a diagnosis of hypergonadotropic hypogonadism.

DISCUSSION

Clinicians should first consider pregnancy when evaluating women of childbearing age experiencing irregular or complete cessation of menses after a normal puberty and menarche. Once this diagnosis has been excluded, the most common endocrine causes of secondary amenorrhea include polycystic ovary syndrome (PCOS) [evaluated by serum testosterone and/or dehydroepiandrosterone sulfate (DHEAS), ovarian ultrasound, and clinical features such as hirsutism], hypothalamic dysfunction (evaluated by FSH measurement), thyroid disease (evaluated by measurement of TSH and thyroid autoantibodies), hyperprolactinemia (evaluated by measurement of prolactin), or primary ovarian insufficiency (POI) (evaluated by assessment of estrogen status and measurement of prolactin, TSH, and FSH) (1) (Fig. 1). Less common causes include Cushing syndrome and nonclassical congenital adrenal hyperplasia. Although algorithms are helpful to distinguish among etiologies, endocrine testing is typically performed simultaneously. If testosterone, TSH, and prolactin are within reference intervals and FSH concentrations are consistently increased, a diagnosis of POI can be established (2, 3). In contrast to...
Menopause, which typically occurs around age 50 years, women with POI (secondary amenorrhea and hypergonadotropic hypogonadism in women younger than 40 years) may experience intermittent follicle development and ovulation. This can lead to spontaneous pregnancy in 5%–10% of women with a diagnosis of POI (4). Approximately 1% of women in the general population experience POI, with an estimated 1.6 mil-

**Fig. 1. Algorithm to guide the diagnosis of women with secondary amenorrhea.**

Multiple laboratory test results must be evaluated, because no single test is diagnostic of the distinct etiologies of secondary amenorrhea. Increased serum FSH, decreased serum estradiol (either measured directly or indicated by a lack of bleeding following a progestin challenge test), and an FMR1 CGG repeat region in the premutation range are diagnostic of FXPOI. *, Estrogen and progestin cycle: estrogens are administered for approximately 21 days to stimulate endometrial proliferation followed by progesterone administration on days 17–21 to induce withdrawal bleeding. †, End Organ Deficiency refers to uterine unresponsiveness to estradiol and progesterone stimulation. ‡, PCOS diagnosis is made according to the Androgen Excess Society Criteria (hyperandrogenism defined by hirsutism and/or increased serum testosterone/DHEAS concentrations, ovarian dysfunction defined by oligomenorrhea/amenorrhea and/or polycystic ovaries on ultrasound and the exclusion of other androgen excess or related disorders). Women undergoing this workup are already experiencing amenorrhea; therefore, additional evidence of ovarian cysts is not required for a diagnosis of PCOS. Bolding in the figure indicates amenorrhea etiology.
lion women affected in the US. Ninety percent of POI cases are idiopathic and the remaining 10% of cases are due to ovarian autoimmunity, exposure to chemotherapy, or genetic causes. Fragile X (FX) premutations constitute the most common genetic cause of POI in women with a normal 46,XX karyotype, with up to 3% of sporadic cases of POI attributed to FXPOI. FX disorders are caused by expansions of the CGG repeat region in the 5′ untranslated region (UTR) of the X-linked fragile X mental retardation 1 (FMR1) gene, which encodes FX mental retardation protein (FMRP), an RNA binding protein involved in regulation of the translation of specific mRNAs. Expanded CGG repeat regions cause a range of pathologic conditions, and the clinical presentation of these disease states depends on the length and methylation status of the CGG repeats (Fig. 2).

Resolution of Case
This patient was referred to a reproductive endocrinologist for further evaluation and management. Because her FSH concentration was increased in the setting of hypoestrogenism, PCR testing of the FMR1 5′ UTR identified the patient as having an FMR1 premutation allele with 82 CGG repeats and the second allele containing 51 CGG repeats (reference intervals: normal, 5–44 repeats; intermediate, 45–54; premutation, 55–200; full mutation, >200 repeats), resulting in a conclusive diagnosis of FXPOI. If the patient’s FMR1 results had been within the reference interval, additional testing would have included evaluation of antiadrenal antibodies and antithyroid antibodies, screening for diabetes, and evaluation of karyotypes.

Importantly, although FMR1 CGG repeats are implicated in both FX syndrome (FXS) and FXPOI, the mechanisms of disease are very different. In individuals with a full mutation, >200 CGG repeats are present and an epigenetic shift from histone acetylation to methylation is observed throughout the repeat region. As a consequence, FMR1 gene expression is silenced and the intellectual disability and facial abnormalities characteristic of FXS arise owing to a lack of FMRP in neurons and other cell types (5). Although the patient’s brother had not been tested at the time of this report, FXS could certainly explain his history of developmental delay. In individuals with a premutation, 55–200 CGG repeats are present but histones in the repeat region remain acetylated and FMR1 overexpression is observed. The prevailing model indicates that this increased concentration of FMR1 mRNA contributes to POI by sequestering RNA binding proteins and thereby interfering with normal cellular processes, particularly in the ovary. Fortunately, only 12%–28% of women with FMR1 premutations experience POI (6).

The mechanisms responsible for repeat expansion are incompletely understood and represent an area of active investigation. Importantly, repeat expansion and contraction is not observed in somatic cells but is strictly associated with the meiotic process. Repeat expansion from a premutation (55–200 repeats) to a full mutation (>200 repeats) has been observed only during maternal transmission, indicating the involvement

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**Fig. 2. Schematic representation of the CGG repeat region in the 5′ UTR of FMR1.**

Repeat length, accompanying epigenetic changes, effect on FMR1 mRNA expression, and association with clinical syndrome are indicated. Ac represents histone acetylation, whereas Me represents histone methylation.
of an oocyte-specific factor in the repeat expansion process. Furthermore, the risk of expansion from a premutation to a full mutation increases with increasing maternal repeat size (5). Genetic counseling is recommended for women with a premutation to determine the probability of expansion to a full mutation upon transmission to their children. One factor that modulates the stability of FMR1 CGG repeats is the frequency of AGG interspersions within the CGG repeat track. It has been observed that AGG repeats serve as stabilizing anchors that prevent uncontrolled expansion.

Two predominant disease models have been proposed to explain FXPOI. The first model suggests a prenatal reduction in the developing oocyte pool in premutation carriers, resulting in exhaustion of ovarian capacity at a younger age following normal follicular maturation and ovarian function. The second model describes a normal oocyte pool characterized by increased rates of follicular atresia owing to the toxic effect of FMR1 mRNA accumulation. Studies of mice with Fmr1 premutations support the second model, because mouse premutation carriers exhibit a primordial follicle pool similar to that of wild-type littermates but experience faster follicular loss (7, 8).

DIAGNOSIS OF FXPOI

Conclusive diagnosis of FXPOI requires confirmation of increased serum FSH concentrations and detection of 55–200 CGG repeats in the 5’ UTR of FMR1. Most clinical laboratories offering FMR1 testing carry out targeted mutation analysis by PCR to investigate the number of CGG repeats. Selected laboratories also assess methylation status or offer complete gene sequencing to identify sequence variants outside the CGG repeat region. Importantly, traditional PCR is highly sensitive when a small number of CGG repeats (≤120) is present but often fails to amplify full mutations, requiring assessment by methylation-specific Southern blot or multiplex ligation-dependent probe amplification. Newer methods are aimed at overcoming this limitation.

CASE FOLLOW-UP

The patient was referred to a genetic counselor for discussion of her risk for having children with FXS and the patient’s family members were encouraged to undergo testing to determine their FMR1 CGG repeat alleles. The patient’s reproductive endocrinologist discouraged in vitro fertilization because in vitro fertilization with autologous oocytes is rarely successful in women with a history of amenorrhea and consistently increased FSH concentrations. In exploring alternative options for childbearing, the patient suggested having her younger sister serve as an oocyte donor. Additionally, preimplantation genetic diagnosis of the resulting embryos was proposed to select an embryo with a minimum number of CGG repeats if the patient’s sister were to test positive for an FMR1 premutation (9). The patient’s sister subsequently underwent clinical evaluation, including FMR1 testing and measurement of serum antimullerian hormone (AMH), an indicator of ovarian reserve (10). The AMH value was 1.8 ng/mL (reference interval for females 13–45 years old, 0.9–9.5 ng/mL), indicating that a standard gonadotropin stimulation protocol would suffice and the patient’s sister could likely serve as an oocyte donor.

POINTS TO REMEMBER

- Initial evaluation of women with oligomenorrhea/amenorrhea should include measurement of testosterone, TSH, prolactin, and FSH.
- Expansions of the CGG repeat region in the 5’ UTR of FMR1 cause a range of pathologic conditions, with the clinical presentation dependent on the number of CGG repeats.
- Diagnosis of FXPOI requires detection of an expanded CGG repeat region in the premutation range, by PCR, sequencing, or Southern blot.
- FXPOI constitutes the most common genetic cause of POI, resulting in 3% of sporadic POI cases.
- Serum AMH concentrations reflect ovarian reserve and can be helpful in assessing potential response to gonadotropin stimulation.

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Commentary

Lawrence M. Nelson*

This case report is about a 33-year-old woman who presented with 5 months of amenorrhea after discontinuing the use of oral contraceptives. I have a different perspective on how to evaluate amenorrhea.

First, I would like more information about the clinical situation. We are told about her medical and surgical history but we are given no information about what else is going on in her life right now. I would have preferred to have the pertinent negatives on the review of systems. Could the amenorrhea be the earliest manifestation of a decline in general health status such as uncontrolled diabetes or other condition? Does she have headaches or symptoms of galactorrhea? What are her eating and exercise habits? Is she having symptoms of estrogen deficiency? How is her emotional health? Did she stop the oral contraceptives because of a desire to get pregnant?

There are no physical findings to suggest androgen excess. A pregnancy test was negative.

Second, I prefer a different approach to the laboratory evaluation. The minimal testing should include the measurement of serum prolactin, FSH, and thyrotropin (recommended by the Practice Committee of the American Society of Reproductive Medicine). In my view the progesterin challenge test is outmoded. I recommend going straight to measuring FSH in this clinical situation and skipping this “ritual.” The progesterin challenge test has not been validated as a test for POI. On the basis of our experience, it certainly must have a high false-negative rate. Most women with POI have intermittent and unpredictable ovarian function. At any one time they may be producing enough estradiol to lead to a withdrawal bleed, but nevertheless at clearly increased serum FSH concentrations. The progesterin challenge test puts women with POI at risk of a significant delay in diagnosis.

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