Reproductive and Gynecologic Care of Women with Fragile X Primary Ovarian Insufficiency (FXPOI)

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Abstract

Objective—Approximately 20% of women with a premutation in the \textit{FMR1} gene experience primary ovarian insufficiency (POI). We explored diagnostic patterns, frequency of appropriate hormone replacement, obstetric outcomes, fertility treatment, reproductive decisions, and counseling of women with fragile X- associated POI (FXPOI).

Methods—Semi-structured interviews with 79 women with FXPOI were conducted by a single interviewer. \textit{FMR1} CGG repeat size was determined from a blood, saliva, or buccal sample.

Results—The median age of POI onset for women in our study was 33 years. 72% of the women had a \textit{FMR1} CGG repeat length of 80–100. Mean length of time from symptom onset to POI diagnosis was 1.12 years, longer in women with a younger age of POI onset and shorter in women who knew they were carriers. After diagnosis, 52% of women never took hormone therapy, started it years after POI diagnosis, or stopped it prior to 45 years of age. 49% of the women had infertility, but 75% had had at least one genetically- related child. Obstetric outcomes were similar to the general population. 46% of women had a diagnosis of low bone mineral density or osteoporosis, and an additional 19% had never had a bone density assessment.

Conclusions—Women with FXPOI are at significant risk for delayed POI diagnosis and undertreatment with hormone therapy. Although nearly 50% of the women had infertility, most were able to conceive at least one child and had no elevated risk of adverse obstetric outcomes.

Keywords

FXPOI; \textit{FMR1}; primary ovarian insufficiency; hormone therapy; infertility

Introduction

Primary ovarian insufficiency (POI), or amenorrhea in the setting of hypergonadotrophic hypogonadism in women younger than 40 years of age, occurs in 1% of women.\(^1\) A
premutation in the FMR1 gene is responsible for 2–11% of POI in the white population, accounting for one of the largest known inheritable causes of POI. The FMR1 gene, located on the X chromosome, normally has a 5’ untranslated region with less than 45 cytosine-guanine-guanine (CGG) trinucleotide repeats. Individuals who carry the “premutation” have between 55–200 copies of the CGG repeat, increased transcription of the FMR1 mRNA transcript, and reduced protein product, FMRP. The large repeat tract in the mRNA is thought to contribute to the clinical findings associated with the premutation. These repeat alleles are also unstable when passed to the next generation and have a risk of expanding to a full mutation, or alleles with greater than 200 repeats. This expansion to >200 repeats results in hypermethylation of the FMR1 regulatory region, absent FMRP protein production, and the clinical disorder of fragile X syndrome (FXS), the most common single gene cause of intellectual disability and autism.

The carrier prevalence for the FMR1 premutation is estimated to be approximately 1/200 women, though there is a wide reported range (e.g. 1/148 to 1/257 women). These women are not only at risk for POI (termed fragile X-associated POI (FXPOI), which occurs in approximately 20% of carriers, but also for diminished ovarian reserve, infertility, poor response to ovarian stimulation, and irregular cycles. In addition to the reproductive impact, women with early menopause have increased risk for earlier mortality, cardiovascular disease, earlier cognitive decline, and osteoporosis. Although these medical risks are at least partly attenuated with estrogen replacement if given prior to the natural age of menopause, women with early menopause are often undertreated with hormone replacement. In addition, they often have an extended time to diagnosis and require multiple visits to healthcare practitioners prior to diagnosis.

Among etiologies of POI, FXPOI is unique since mutations in the FMR1 gene manifest in other family members, either as FXS or Fragile X-associated tremor/ataxia syndrome (FXTAS), potentially shortening the time to diagnosis of POI. In addition, women with the premutation struggle with reproductive decisions involving the possibility of not only impending POI but also the risk of passing the pre- or full mutation to their offspring. The objective of this study was to explore POI diagnostic patterns, the frequency of appropriate hormone therapy (HT), obstetric outcomes, fertility treatment, and counseling of women with FXPOI. In addition, we examined the reproductive decisions of women with the premutation made after they are diagnosed as carriers.

Methods

Participants

The study population was ascertained from two different sources. First, 71 of the women with FXPOI were identified from a previously described large cohort of women with the premutation. This cohort has been gathered from families with individuals with the fragile X pre- or full mutation through fragile X clinics, fragile X syndrome parent support groups and listservs, and conference recruitment since 2000. Information from a previously filled out a reproductive history questionnaire, which included demographic information, age of last menstrual period, information regarding use of hormone therapy (e.g. oral contraceptives, hormone replacement, etc.), and parity, was used to identify 89 women as
possibly having had POI. This was narrowed to 71 women after detailed interviews. Eight additional women were ascertained during a nationwide search for women with FXPOI as part of a larger study identifying genetic variants that influence the development of FXPOI. These women were recruited from collaborators at fragile X clinics and a fragile X syndrome listserv. From these two sources, 79 women with the premutation were identified with FXPOI.

**Data Collection and Variable Definitions**

Although POI has several proposed definitions, it was defined for the purposes of this study as secondary amenorrhea of at least 4 months duration in the setting of menopausal FSH levels.\(^{25}\) Diagnosis was based on self-report; medical records were not obtained to verify laboratory values. The exclusion criteria were non-English speaking and age older than 75 years at time of interview.

Demographic information was collected, including date of birth, race, education level, and current city of residence. The cities were classified by United States (US) region (South, Northeastern, Midwestern, and Western) and then by urban versus rural, as defined by the US census.\(^{26}\) Semi-structured telephone interviews were conducted by a single interviewer, a reproductive endocrinologist and board certified Obstetrician and Gynecologist (HH). The interviews lasted approximately 15 to 30 minutes and took place over 8 months, from January to August of 2015. The women were queried regarding their obstetric history and any pregnancy or postpartum complications. They were asked to recall the narrative of their POI diagnosis, including the length of time to diagnosis and number of healthcare providers seen. Data were also collected regarding history of infertility and reproductive treatment, medical conditions, menopausal symptoms, and hormone therapy (HT) use. Their HT use was defined as “sub-optimal” if they never took HT, if they took HT for less than one year, if they stopped HT less than 45 years of age, or if they started HT more than a year after their POI diagnosis.\(^{25,27,28}\) Lastly, the women were asked about their reproductive decisions after detection of their \(FMR1\) premutation. The protocols and consent forms were approved by the Emory IRB.

**\(FMR1\) repeat size assay**

All women provided either a buccal swab, saliva, or blood sample to determine repeat size. DNA was extracted using Qiagen QiAmp DNA Blood Mini Kit. \(FMR1\) CGG repeat sizes were determined by a fluorescent-sequencer method, as described elsewhere,\(^{29}\) using the ABI Prism 377 DNA Sequencer or ABI Prism 3100 Genetic Analyzer. If only one allele was identified, a second PCR-based, hybridization technique was used to identify a possible high band, a modified protocol based on prior publications.\(^{30}\)

**Statistics**

We calculated descriptive statistics, reporting results as median +/- standard deviation and a range. Means between groups were compared using a student’s t-test. Frequencies were compared using Chi-square tests. The influence of US region was tested using an ANOVA model with a Tukey’s post-hoc test to compare different regions. Linear regression and logistic regression were used to investigate variables that significantly contribute to the time
Results

Demographics of the study population are presented in Table 1. The median age at time of interview was 48 years (range 29–75). At the time of the interview, 0–40 years (median 16) had elapsed since the diagnosis of POI. The median age of POI was 33 years (range 18–39). The majority of the women (83.5%) learned they were carriers after a family member was diagnosed with FXS. The remaining women were tested after a personal or family history of POI (15.2%) or after preconception carrier screening (1.3%). Among all the women, 54.4% had children with FXS.

Time to diagnosis of FXPOI

Figure 1 illustrates the length of time to diagnosis of POI by the year that women were diagnosed with POI. There was no improvement over the last 3 decades. Mean amount of time to diagnosis from the time women first identified concerns to a healthcare provider was 1.12 years (range 0–13). For women who knew they were premutation carriers after FXPOI had been described (12 women), their mean time to diagnosis was 0.76 years (range 0–4). Women saw an average of 2 healthcare providers (range 0–7) to obtain a diagnosis. Only three women reported difficulty accessing a physician as a barrier to diagnosis. To investigate possible barriers that contributed to an increased time to diagnosis, we first tested a linear regression model with time to diagnosis as the outcome. The predictor variables tested included age at POI diagnosis, whether they knew their carrier status prior to POI onset, and year of diagnosis of POI. Of the predictor variables tested, only age at POI diagnosis was significant (p=0.03, beta coefficient −0.11, 95% CI −0.21 to −0.01). We also dichotomized time to diagnosis to compare women who received a diagnosis within one year or less compared to those women who took more than one year to receive a diagnosis. In a logistic regression model comparing these two groups, the predictor of age at POI diagnosis was again the only significant risk factor (adjusted OR=0.86; 95% CI: 0.76–0.97). In short, women who were younger at time of POI diagnosis were more likely to have a longer time to diagnosis. The year in which women were diagnosed, the area of country in which they lived, living in an urban versus rural environment, and education level did not significantly impact the lag time.

During their climacteric transition, 77% of the women experienced recurrent vasomotor symptoms such as hot flashes or night sweats. When they initially presented to their physician, 25.3% of the women recounted that their symptoms and amenorrhea were discounted, delaying their diagnosis. Five of these women knew they were carriers and at risk for FXPOI.

Hormonal treatment

In regards to HT use, 24 women reported never having taken HT or having taken it for less than a year. Fifteen women started HT more than a year after a diagnosis of POI. Three women stopped prior to 45 years of age. All together, 41 women (51.8%) had “sub-optimal”
HT use. Of these women, 23 said their doctors never mentioned HT or advised them against using HT. The remainder disliked the side effects (3 women), disliked taking medications (5 women), did not have monetary resources for medication (1 woman), did not notice any benefit (1 woman), or were concerned about long term health risks especially in regards to the media coverage of the Women’s Health Initiative findings (8 women). There was not a significant association between HT use and year of POI diagnosis, area of country, rural vs. urban environment, or education level.

**Infertility Treatment**

In regards to infertility among the women interviewed, 49.3% either used fertility treatment or had unsuccessful attempts at conception for more than a year. Of those who used fertility treatment, eighteen underwent super-ovulation (e.g. with clomiphene citrate, letrozole, or injectable gonadotropins) in combination with timed intercourse or intrauterine inseminations. Three underwent in- vitro fertilization (IVF) with autologous oocytes and seven with donor oocytes. No women did IVF with pre-implantation genetic diagnosis. After learning of their FMR1 premutation status, six women continued to pursue fertility treatment with autologous eggs (with either oral or injectable medications), though none conceived.

**Obstetric History and Outcomes**

Among all the women in this study, 17.7% women were nulliparous or had only non genetically-related children using oocyte donation. The majority of the women (74.6%) had at least one genetically- related child (median= 2 children, range 1–4). In regards to obstetric losses, 24.0% women had experienced at least one miscarriage, with only one woman who had had more than two. Two women had spontaneous dizygotic twins. Out of all the children born to women in this study (133), 6.0% were born premature, at less than 37 weeks gestation. Of the deliveries, 15.0% were cesarean sections. During their pregnancies or postpartum period, 6.3% of the women had pre-eclampsia, 3.8% had gestational diabetes, and 16.4% reported symptoms of postpartum depression. Median maternal age at delivery of all children (with autologous oocytes) was 28 years (range 17–42) and median maternal age at delivery of last (youngest) child was 30 years (range 19–42).

Among women diagnosed with FXPOI, ten (12.6%) conceived spontaneously after they were diagnosed with POI and four of the women conceived ≥ 2 times for a total of 15 pregnancies. Three of the women were ≥38 years old at delivery. The interval of time to conception after diagnosis of POI ranged from 0 years to 12 years. Out of the 15 pregnancies, 12 resulted in full-term pregnancies and the remainder were terminated.

**Reproductive Decisions after Diagnosis of Premutation Carrier Status**

After learning of their premutation carrier status, 62% of the women said knowledge of their carrier status affected their reproductive decisions. Out of these 49 women, 40 stopped having children or reported they would have stopped had they known they were carriers during their reproductive years. Five women used an oocyte donor with in vitro fertilization to avoid passing along the FMR1 mutation. Two women had hysterectomies as birth control, both reporting they wanted something more “definitive” than a tubal ligation. Two women terminated pregnancies to avoid passing on the mutation. Of the other women, 24.0% said
their reproductive and childbearing decisions were not affected by knowledge of their carrier status and 13.9% of the women were unsure if knowledge of their carrier status affected their reproductive decisions. Many of them felt very conflicted, reporting the joy their child with FXS brought to their family combined with a reluctance to knowingly pass along a disabling gene.

Co-occurring Medical Conditions

In regards to medical conditions, 20.2% of women had thyroid disease, 26.5% had a history of a mood disorder, such as anxiety or depression, and 8.8% had hypertension. 46% of the women had a diagnosis of osteoporosis or low bone mineral density (BMD) with an additional 18.9% of women who had never had a bone density assessment. Of the women who had not had a bone density assessment, 66% of them reported their physicians never mentioned the risk of low bone density associated with a hypoestrogenic state. The other women had had infrequent or absent follow-up with their physicians. A diagnosis of osteoporosis or low BMD was associated with increasing length of time to POI diagnosis, but this was not significant (crude OR = 1.29, 95% CI = 0.93–1.79; p = 0.07). Likewise, having “sub-optimal” HT use increased the odds of having osteoporosis or low BMD, but it was not significant (crude OR = 1.98, 95% CI = 0.73–5.35; p = 0.17). This was un-changed if model was adjusted for age and the number of years to diagnosis. Of note, 47% of women with acceptable HT use developed osteoporosis or low BMD as compared to 64% of women with sub-optimal HT use.

DISCUSSION

In our study, 72% of the women with FXPOI had a repeat size length of 80–100 CGG repeats, similar to findings from prior studies that identified women with 80–100 repeats to be at the highest risk for FXPOI compared to repeat lengths 59–79 or >100. Based on mouse models, increased transcription of the FMR1 mRNA transcript with the large repeat track is thought to cause ovarian function toxicity, though the etiology behind the curvilinear repeat dose and toxic effect is still unknown. A recent review summarized mechanisms through which the long repeat tract in the mRNA possibly affects ovarian function: sequestration of CGG- binding proteins that are crucial to cell viability; formation of secondary RNA structures, such as hairpins, that act as substrates for enzymes that generate small RNAs that interfere with gene expression; transcription of anti-sense transcripts that anneal to sense transcripts and activate the immune system; or alteration of translation start codons that results in toxic protein formation.

We found it took women with FXPOI a mean of about one year to be diagnosed with POI from the time they initially presented with to a provider with symptoms. Few women reported difficulty accessing healthcare and time to diagnosis would presumably be longer in women with low healthcare access. This is similar to data from a 2002 study that reported a delay of two years for POI diagnosis and that 25% of women waited more than five years for a diagnosis. The women in our study who knew they were carriers at the time of POI onset had a shorter time to diagnosis than the remainder of the women. During the interviews, several women who knew their carrier status reported printing out information from fragile
X websites and taking it to their Gynecologist after their initial concerns were discounted. Ideally, a diagnosis of an offspring with FXS should not only prompt a referral to a genetic counselor to discuss inheritance patterns, but also, reflexively, a discussion regarding the health risks for premutation carriers, including FXPOI.

Any delay in diagnosis or under-prescribing of HT may increase the risk of comorbidities of POI. These may be partially attenuated with estrogen replacement, highlighting the need for early diagnosis and appropriate hormone replacement. In women with POI, estrogen has been shown to preserve bone density\textsuperscript{36} and improve vascular endothelial dysfunction,\textsuperscript{37} a risk for cardiovascular disease. More than half of the women in our study had sub-optimal HT use, with the majority reporting their doctor never mentioned HT or advised them against using HT. Little has been written about HT in women with POI or those with FXPOI, though these findings are similar to a large Swedish epidemiologic study that found HT is likely under-prescribed to women who undergo menopause prior to 45 years of age.\textsuperscript{22} As others have noted,\textsuperscript{25,27} the conclusions of the Women’s Health Initiative,\textsuperscript{31} which was conducted in older woman, do not extend to women with POI. There is an age window during which HT is beneficial, and its use is recommended by The North American Menopause Society until the median age of natural menopause.\textsuperscript{28}

In that regard, almost half of the women in our study had a diagnosis of osteoporosis or low bone mineral density and an additional 20% had never undergone a bone density assessment. Although our association of sub-optimal HT and osteoporosis was not significant, un-treated women with POI have been found to have significantly decreased bone mineral density (BMD) compared to age-matched controls,\textsuperscript{38,39} with discrepancies that remain into their 60s.\textsuperscript{20} Anasti et al. found 67% of women with POI to have BMD scores more than 1 standard deviation below the mean for age-matched women, placing them at a 2.6-fold increased risk for hip fracture. Half of the women had low BMD scores within 1.5 years of diagnosis of POI.\textsuperscript{21} In addition, for women with POI at young ages, peak bone mass, which typically occurs at 30 years of age, is not achieved in the setting of early hypoestrogenism. Optimal HT, which 50% of the women in our study did not receive, can likely attenuate POI’s impact on bone mineral density.\textsuperscript{36}

The obstetric and parity data in our study are encouraging for women with FXPOI. 75% of the women had had at least one child without intervention, though this is possibly an overestimate for the general population of women with FXPOI. Many of the women were ascertained because they had a child with FXS, thus proven fertility. The miscarriage risk was similar to that of the general population.\textsuperscript{40} Obstetric risks were similar to or lower than those in large epidemiologic studies for pre-eclampsia,\textsuperscript{41} gestational diabetes,\textsuperscript{42} prematurity,\textsuperscript{43} and cesarean section.\textsuperscript{43} Our population did, however, start having children in 1960 when the prevalence of all these obstetric morbidities were lower. We found a lower risk of pre-eclampsia than other studies in women with FXPOI,\textsuperscript{13} but our results are similar to an older study that concluded carriers were not at increased risk for adverse obstetric outcomes except for a slight risk for non-specific late pregnancy bleeding.\textsuperscript{44}

The average maternal age at delivery (28 years) for women in our study is similar to United States data.\textsuperscript{43} Of the women with FXPOI who had children, however, 93% completed

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childbearing by 35 years or younger. As more women choose to delay childbearing, as illustrated by a gradually increasing maternal age at first delivery in the United States, women who carry the premutation will likely increasingly be diagnosed with POI prior to childbearing. Already, approximately 50% of women in this study had infertility, much higher than the general population. This may be an underestimate given that many women were ascertained because they had a child with FXS. Women identified as carriers in the early part of their reproductive lifespan should be monitored closely and, if desired, referred to a reproductive specialist to discuss family planning. Women at risk for early ovarian aging can be offered embryo or oocyte cryopreservation for fertility preservation with caveats regarding lack of outcome data in this population.

Ten of the women in our study conceived after a diagnosis of POI, with a lag time of up to 12 years after initial diagnosis. This percent (12.6%) is incrementally higher than that reported in prior studies, which report a 5–10% spontaneous conception rate following a diagnosis of POI. This increased frequency may be due to the fact that six of the women conceived within one year of POI diagnosis, likely attributable to fluctuating residual ovarian function many women have after diagnosis.

Following diagnosis of their premutation carrier status, women in this study made a wide variety of reproductive choices. Our findings are similar to other studies that found that about 60% of women decide to stop having genetically-related children after learning of their carrier status, while 25% of women do not change their reproductive plans. Women in our study who did not change their reproductive plans were less likely to already have a child with FXS (36.8%) than the women who did change their plans (62%). There have been significant advances in reproductive medicine that allow childbearing while preventing transmission of potentially morbid genetic diseases, including IVF with pre-implantation genetic diagnosis or oocyte/embryo donation. Women who carry the premutation would benefit from counseling that includes this information.

The incidence for thyroid disease (20.2%) in women with FXPOI in our study is only slightly higher than found in other studies in women with premutation, with rates between 10–17%. This is likely based on sample size alone, though women with spontaneous POI are at higher risk for thyroid disease. The risks for mood disorders are similar to those in other studies.

Strengths of our study include a relatively large population of women diagnosed with FXPOI, using a consistent definition. Other studies have included less than 75 women and use survey methodologies. The structured interview by a reproductive endocrinologist allowed us to gather a wider and more detailed amount of information about aspects of care, including details regarding obstetric history and the use of HT and fertility medications, that other studies have not evaluated. We were able to capture women with a range of educational backgrounds, with 37% not having completed college, and from all areas of the country, including a mix of both urban and rural areas. Lastly, we verified premutation carrier status with CGG repeat testing.
Our study is limited in its inclusion of mostly white women of Northern European ancestry despite nationwide recruitment, a limitation found in other fragile X literature. African Americans, in particular, are known to be under-represented in fragile X research studies, which is thought to be secondary to delay in diagnosis of fragile X and communication issues within families and with healthcare professionals. Women with low healthcare access are also under-represented as the sample was not recruited from the general population, but from families who had access to a diagnosis of fragile X full- or premutation. These selection biases likely result in us underestimating lapses in gynecologic care for women with FXPOI. Lastly, medical history was collected by self-report instead of medical record review and therefore cannot be validated. We excluded women who were unsure about or could not remember aspects of their medical history.

Conclusion

In summary, we have characterized reproductive and gynecologic history and care for a relatively large cohort of women with FXPOI. Their obstetric risks are similar to the general population. Importantly, however, ovarian insufficiency places these women at increased risk for infertility, undertreatment with HT, and other diseases associated with a hypoestrogenic state, such as osteoporosis. Increased education about POI and guidelines for HT treatment can decrease diagnosis time and likely attenuate long term health risks for women with FXPOI.

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References


Figure 1.
Length of time (Years) to diagnosis of POI by year that women were diagnosed with POI shows no improvement over last 3 decades.
Table 1

Demographics of Interviewed Study Participants with FXPOI

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