Increased frequency of occult fragile X-associated primary ovarian insufficiency in infertile women with evidence of impaired ovarian function


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BACKGROUND: The FMR1 premutation is associated with overt primary ovarian insufficiency (POI). However, its prevalence in women with occult POI (i.e. menstrual cycles, but impaired ovarian response) has not been examined. We hypothesized that both the FMR1 premutation and intermediate allele is more frequent in infertile women with occult POI than in controls, and that a repeat length cutoff might predict occult POI.

METHODS: All subjects were menstruating women <42 years old and with no family history of unexplained mental retardation, autism or fragile X syndrome. Cases had occult POI defined by elevated FSH or poor response to gonadotrophin therapy (n = 535). Control subjects (n = 521) had infertility from other causes or were oocyte donors. Prevalence of the FMR1 premutation and intermediate alleles was examined and allele length was compared between controls and women with occult POI.

RESULTS: The frequency of the premutation (7/535 versus 1/521; P < 0.05) and intermediate alleles (17/535 versus 7/521; P < 0.05) was higher in women with occult POI than in controls. The allele with the greatest number of CGG repeats was longer in women with occult POI compared with controls (32.7 ± 7.1 versus 31.6 ± 4.3; P < 0.01). A receiver operating characteristic curve examining repeat length as a test for occult POI had an area of 0.56 ± 0.02 (P < 0.01). A repeat cutoff of 45 had a specificity of 98%, but a sensitivity of only 5% to identify occult POI. The positive predictive value was only 21% for a fertility population that has ~22% of its patients with occult POI.

CONCLUSIONS: The data suggest that FMR1 premutations and intermediate alleles are increased in women with occult POI. Thus, FMR1 testing should be performed in these women as some will have fragile X-associated POI. Although the FMR1 repeat lengths were longer in women with occult POI, the data do not support the use of a repeat length cutoff to predict occult POI.

Key words: fragile X syndrome / primary ovarian insufficiency / intermediate repeat / infertility / ovarian aging

Introduction

Fragile X syndrome is the most common cause of inherited mental retardation, affecting 1 in 4000 males and 1 in 8000 females (Turner et al., 1996). Fragile X syndrome results from an expansion of the CGG repeat in the 5’ untranslated region of the FMR1 gene. When the triplet repeat number exceeds 200, the gene is methylated and silenced (Fu et al., 1991; Snow et al., 1993). A repeat length of 55–200 is termed a premutation, as it can expand to >200 repeats in progeny through the mother (Nolin et al., 2003).

Women who carry the premutation may develop fragile X-associated primary ovarian insufficiency (FXPOI) (Murray, 2000; Murray et al., 2000; Hunter et al., 2008). Primary ovarian insufficiency (POI) is a term that encompasses the spectrum of ovarian dysfunction, and describes the severity of ovarian dysfunction through modifying terms (Welt, 2007). Women with decreased fecundity despite...
regular menses as a consequence of decreased ovarian reserve have occult POI. Those with regular menses but an elevated FSH or low inhibin B or Anti-Mullerian Hormone (AMH) have biochemical POI, a subset of occult POI. Women with irregular or absent menses and an elevated FSH before the age of 40 years have overt POI, previously termed premature ovarian failure (POF) (Welt, 2007). POI in all degrees of severity has been identified in women who carry the premutation, and FXPOI is the preferred term to describe the ovarian dysfunction associated with fragile X (McConkie-Rosell et al., 2007).

The association between premutation status and overt POI is well established. Approximately, 20% of women who carry the premutation experience overt POI, in contrast to only 1% of women in the general population (Conway et al., 1998; Murray et al., 1998; Marozzi et al., 2000; Mallolas et al., 2001; Bussani et al., 2004). In addition as a group, premutation carriers experience earlier menopause by ~5 years (Sullivan et al., 2005; Ennis et al., 2006). There is also evidence for occult POI (subgroup biochemical POI) in women carriers between 18 and 40 years who have regular menstrual cycles, elevated FSH levels and decreased inhibin B and AMH levels (Murray et al., 2000; Hundscheid et al., 2001; Welt et al., 2004; Rohr et al., 2008). Further occult POI, manifested as decreased fecundity with normal menstrual cycles, is suggested by the increased rate of infertility in women carriers compared with non-carriers (Allen et al., 2007).

Based on the broad range of ovarian dysfunction in women with FXPOI, these women may be identified when they present in an infertility evaluation. While the relationship between FMR1 premutations and overt POI is well established, the prevalence of the FMR1 premutation in women presenting with occult POI, also termed decreased ovarian reserve, has not been examined.

There is also controversy regarding the repeat length associated with ovarian dysfunction. Of note, the FMR1 premutation repeat length (55–200 repeats) was defined based on the likelihood that the repeat will expand to ~200 (full mutation) in the fetus, resulting in fragile X syndrome. The premutation can expand to a full mutation within one generation, whereas the intermediate repeat length in fragile X syndrome. The premutation can expand to a full mutation and the repeat will expand to full length. The current cutoff length (55–200 repeats) was defined based on the likelihood that an intermediate repeat length (35–44 repeats) are associated with overt POI (Bretherick et al., 2005; Bodega et al., 2006). Intermediate expansions were enriched in women with overt POI compared with women with a normal age at menopause (Bretherick et al., 2005; Bodega et al., 2006). However, a large UK study did not find evidence for an association between intermediate size repeats and overt POI (Bennett et al., 2010). The relationship between intermediate size repeats and occult POI also needs examination. Further, given that the definition of premutation and intermediate alleles is related to the potential for expansion and not ovarian function, a lower limit cutoff for the repeat length associated with ovarian function needs to be determined.

This study was designed to test the hypothesis that the prevalence of the FMR1 premutation and intermediate alleles is increased in women with occult POI, before the development of overt POI. It was also designed to determine whether there was a repeat length cutoff that could be used to predict occult POI. In addition, while it is recommended by the American College of Medical Genetics and/or the American College of Obstetrics and Gynecology that women with an elevated FSH and/or infertility or ovarian insufficiency consider FMR1 testing (Sherman et al., 2005; ACOG, 2010), it is not clear whether these recommendations are being followed. Therefore, the study also determined the level of FMR1 repeat screening performed in women since guidelines were put into effect. Three fertility practices performing over 50% of the IVF cycles in the metropolitan Boston area participated in the study, representing the largest cohort examined to date.

### Materials and Methods

The study subjects were identified using chart review for all women presenting for infertility treatment and with FMR1 testing between January 2006 and December 2010, at Brigham and Women’s Hospital, Massachusetts General Hospital and Boston IVF (Fig. 1). The resulting subject list of women with occult POI was compared with that in databases maintained at the three institutions to ensure that a complete cohort of women with occult POI had been tested for FMR1 repeat number. Women with occult POI who had not been tested were recruited by mailing. A list of subjects failing to respond to the mailing after 3 months and with no family history (see below) was submitted to the Harvard Crimson Pathology Service, which prospectively collected discarded blood samples from these patients (IRB approved), de-identified the samples and provided them for FMR1 testing. All protocols were approved by the Partners Human Research Committee and the Beth Israel Deaconess Human Research Committee and all recruited subjects gave written informed consent.

All cases were women <42 years of age, who met predefined criteria for occult POI and were menstruating (n = 535). In the current study, occult POI was defined as: (i) elevated FSH (>10 IU/l) on cycle day 2–4, on Day 10 of a clomiphene citrate challenge test or at random if cycles were irregular (Nahum et al., 2001; Yanushpolsky et al., 2003; Klipstein et al., 2005); or (ii) normal FSH with an elevated Day 2–4 estradiol (E2) (>80 pg/ml); or (iii) a poor response to gonadotrophin stimulation, defined as less than five follicles of ≥12 mm after treatment with 450 international units (IU) of gonadotrophins per day or more. The subjects would also fit within a liberal categorization of decreased ovarian reserve. Subjects were excluded for amenorrhea greater than 3 months in duration, i.e. overt POI, previous surgical removal of an ovary and chemotherapy or radiation therapy. Subjects were also excluded for a family history of unexplained mental retardation, autism or fragile X syndrome and for an abnormal karyotype associated with infertility. Women who received genetic counseling were noted and the family history was compared with that obtained by the infertility specialists. A negative family history was determined to be consistent between the infertility specialist and genetic counselor if genetic counseling did not reveal a family history of unexplained mental retardation, autism or fragile X syndrome in up to third-degree relatives. Subjects underwent treatment with clomiphene citrate or gonadotrophins, with or without IVF as directed by the subject’s physician. A subset of subjects did not undergo treatment because they were told of a poor prognosis and either did not continue or were directed to treatment with a donor oocyte.

Control subjects (n = 521) were women presenting to the same institutions and over the same time frame as the cases. Controls had infertility with no evidence of occult POI (n = 389) and no suspected or known family history of unexplained mental retardation, autism or fragile X syndrome, or were women presenting as anonymous (n = 120) or directed
oocyte donors \((n=12)\). Control subjects underwent FMR1 screening in the same laboratory and/or using the same methods as study subjects. FMR1 repeat length was determined by PCR. If only one allele size was observed, Southern blot was performed (Esoterix Genetic Laboratories, LLC, Westborough MA or Mayo Clinic, Rochester, MN).

**Statistical analysis**

The main outcome was prevalence of premutation, and intermediate allele sizes in women with occult POI compared with controls. Secondary outcomes were the relationships between repeat number and occult POI and repeat number and the following: Day 3 and Day 10 FSH levels, Day 3 E2 levels, antral follicle count (defined as the number of 3–10 mm follicles in the follicular phase of the cycle before starting treatment), number of stimulated follicles and average gonadotrophin dose per day in subjects undergoing IVF. Secondary outcomes also included the rate of FMR1 screening performed each year.

Sigma Plot version 11.0 software was utilized for analysis. Prevalence of the FMR1 premutation and intermediate size repeat and clinical diagnoses were compared in subjects with occult POI and controls using the \( \chi^2 \) test. Clinical characteristics, the allele with the greatest number of CGG repeats and the allele with the lowest number of CGG repeats in each subject were compared between subjects with occult POI and controls using a Mann–Whitney rank sum test and the relationships between clinical characteristics and repeat length were examined using Spearman correlations. The receiver operating characteristic (ROC) curve was used to determine whether there was a threshold repeat size that could be used to predict occult POI. The rate of FMR1 screening was examined over the years using analysis of variance on ranks. Results are presented as mean ± SE unless otherwise indicated. A \( P \) value < 0.05 was considered significant.

**Results**

FMR1 screening of women with occult POI by the reproductive specialists increased yearly during the years 2006–2009 \((P < 0.05; \text{Fig. 1})\). A positive family history was present in 32 women when the history was taken by reproductive specialists. Genetic counseling added six positive family histories (Fig. 1). A karyotype deviating from 46XX was present in 12 women of 313 (3.8%), while 222 cases were untested. The karyotypes were as follows: 46,XX,t(5;18)(p15.3;q21.3), 46XX t(9;11)(q22;q23), 46XX inv(22)(p11q11.2), 46XX inv(9) (p12q13), 46,XX inv(18)(q21.1q23), 46XX inv(3)(p14p26.2), 46XX 4qs.ish 4q35(Tel4qx2), 47XX, mosaic 46XX and 47XXX and mosaic 46XX with 1–2 cells of 45X. All subjects with karyotype abnormalities associated with infertility were removed from analysis, specifically the mosaic sex chromosome abnormalities (Bugge et al., 2000; Laml et al., 2002).

Women were diagnosed with occult POI on the basis of elevated FSH \((n=254)\), elevated day 3 E2 \((n=104)\), elevated day 10 FSH \((n=40)\) and/or poor response to gonadotrophins \((n=191)\). In addition, they presented with regular cycles (25–35 days; 77%), short cycles (<25 days; 9%) and long cycles (36–90 days; 14%). Additional characteristics of subjects with occult POI and controls are described in Table 1. Women with occult POI were older than the controls and additional infertility diagnoses differed as expected (Table 1). Also as expected by definition, the average FSH levels on Day 3 and on Day 10 after a clomiphene citrate challenge test were elevated in women with occult POI, the antral follicle count was lower and the number of follicles after gonadotrophin stimulation was lower in women with occult POI.
Frequency of the premutation and intermediate alleles was higher in women with occult POI and no family history than in control women (Table II). Further, the allele with the greatest number of CGG repeats was longer in women with occult POI and no family history than in control women (Table II). Further, the allele with the greatest number of CGG repeats was longer in women with occult POI and no family history than in control women. The allele with the greatest number of CGG repeats in the occult POI group to that in controls had an area under the ROC curve of 0.56. At 31.5 repeats, the sensitivity for detecting occult POI was 72% and the specificity was 72% (Table II).

To determine whether there was a repeat size that could be defined to predict occult POI, the ROC curve was examined. An ROC curve comparing the repeat length of the allele with the greatest number of CGG repeats in the occult POI group to that in controls had an area under the ROC curve of 0.56. At 31.5 repeats, the cutoff with the best sensitivity and specificity to detect occult POI, the sensitivity was 72% and specificity was only 36%. Using a cutoff of 35 repeats, the lower limit of the repeat number defined for intermediate carriers in some studies (Bretherick et al., 2005; Bodega et al., 2006; Gleicher et al., 2009a; Bennett et al., 2010), the specificity was 88% and the sensitivity 17%. Using a cutoff of 45 repeats as the lower limit defining intermediates, the specificity was 98%, but the sensitivity was only 5%. Using the lower limit repeat number defined for premutation carriers (55 repeats), the specificity was 99.8% and the sensitivity was 1.6%. If the rate of occult POI in a fertility practice is 22%, which is the rate among the subgroup of patients with decreased ovarian reserve aged 41–42 years (www.cdc.gov/mmwr), an intermediate allele length of 45 had a positive predictive value of 21%, whereas the negative predictive value was 91%. For other age groups, the positive predictive value would be lower as the prevalence of occult POI is also lower.

Treatment cycle characteristics were examined as a function of the longest repeat length. There was no correlation between FMR1 repeat length and Day 3 or Day 10 FSH level, Day 3 E2 level, quantity of gonadotrophin administered, follicle number or antral follicle count (all P > 0.05). There was also no relationship with age, i.e. it was

### Table I  Subject characteristics, hormone levels, gonadotrophin dose and stimulated follicle number in women with POI undergoing fertility treatment and controls.

<table>
<thead>
<tr>
<th></th>
<th>POI</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean ± SD)</td>
<td>36 ± 3 (33 ± 6)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 0.3 (24.5 ± 0.3)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Additional infertility diagnosesa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>NA</td>
<td>70</td>
<td>NA</td>
</tr>
<tr>
<td>Male infertility</td>
<td>78</td>
<td>70</td>
<td>0.3</td>
</tr>
<tr>
<td>Age &gt; 39 years</td>
<td>83</td>
<td>78</td>
<td>0.07</td>
</tr>
<tr>
<td>Recurrent miscarriage</td>
<td>16</td>
<td>51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uterine anomaly</td>
<td>14</td>
<td>25</td>
<td>0.004</td>
</tr>
<tr>
<td>Tubal defect</td>
<td>22</td>
<td>27</td>
<td>0.06</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>18</td>
<td>28</td>
<td>0.008</td>
</tr>
<tr>
<td>PCOS</td>
<td>NA</td>
<td>77</td>
<td>NA</td>
</tr>
<tr>
<td>HA</td>
<td>NA</td>
<td>11</td>
<td>NA</td>
</tr>
<tr>
<td>PGD for genetic Dx</td>
<td>2</td>
<td>9</td>
<td>0.007</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>2</td>
<td>8</td>
<td>0.015</td>
</tr>
<tr>
<td>Unknown</td>
<td>NA</td>
<td>18</td>
<td>NA</td>
</tr>
<tr>
<td>Family Hx POI</td>
<td>13</td>
<td>13</td>
<td>0.4</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>17</td>
<td>20</td>
<td>0.8</td>
</tr>
<tr>
<td>Former</td>
<td>27</td>
<td>21</td>
<td>0.6</td>
</tr>
<tr>
<td>Passive</td>
<td>7</td>
<td>7</td>
<td>0.1</td>
</tr>
<tr>
<td>Day 3 FSH (IU/l)</td>
<td>16.3 ± 0.6 (6.7 ± 0.1)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Day 10 FSH after clomiphene citrate challenge (IU/l)</td>
<td>17.9 ± 1.1 (8.7 ± 0.6)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Day 3 E₂ (pmol/l)</td>
<td>192 ± 10 (158 ± 13)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>5.9 ± 0.3 (4.5 ± 0.5)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Gonadotrophin dose/day in first cycle of treatment (IU)</td>
<td>543 ± 36 (414 ± 45)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Total follicle number ≥ 12 mm diameter on day of hCG administration</td>
<td>6.3 ± 0.41 (10.3 ± 2.0)</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

NA, not applicable; HA, hypothalamic amenorrhea.

### Table II  Prevalence of the FMR1 premutation and intermediate allele in women with POI and in controlsa.

<table>
<thead>
<tr>
<th></th>
<th>Women with POI</th>
<th>Controls</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premutation (55–200 repeats)</td>
<td>7/535 (1.3%)</td>
<td>1/521 (0.19%)</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>Intermediate (45–54 repeats)</td>
<td>17/535 (3.2%)</td>
<td>7/521 (1.3%)</td>
<td>0.046</td>
<td></td>
</tr>
</tbody>
</table>

POI, primary ovarian insufficiency.
a Subjects with positive family history or an abnormal karyotype known to cause infertility are not included.

### Figure 2  Allele size distribution for the allele with the greatest repeat length in women with POI (n = 535; black bars) and controls (n = 521; white bars). The repeat size was longer in women with POI than in controls (P < 0.01).
not younger women who presented with the premutations or intermediate alleles.

**Discussion**

POI is a term that encompasses the spectrum of ovarian dysfunction from decreased fecundity (occult POI) to amenorrhea related to ovarian dysfunction (overt POI). While it is well known that the FMR1 premutation is associated with overt POI, its prevalence in women with occult POI presenting to a fertility practice with a severity equivalent to decreased ovarian reserve had not been fully examined. The present data demonstrate that the prevalence of the FMR1 premutation is increased in women with occult POI compared with women with other causes of infertility and oocyte donors. The frequency of the FMR1 intermediate allele was also higher than in controls in the absence of a family history of mental retardation or autism. Thus, the etiology of occult POI in women who present to an infertility practice may be FXPOI (McConkie-Rosell et al., 2007). Further, the repeat length in the allele with the greater number of repeats is longer in women with occult POI than in controls. However, using FMR1 CGG repeat length in women with infertility as a test to predict occult POI in less severe forms than overt POI has a poor positive predictive value.

The current study demonstrates a relationship between the premutation and occult POI, consistent with the well-documented relationship between the premutation and overt POI (Coulam et al., 1986; Allingham-Hawkins et al., 1999; Murray, 2000; Murray et al., 2000; Sherman, 2000). The prevalence of the premutation was only 1.3% rather than the expected prevalence of 3% if the rates were the same as those in women with overt POI (Allingham-Hawkins et al., 1999). The difference may be related to a large number of women with occult POI related to aging or other risk factors that would dilute the overall prevalence of FMR1 premutations. In addition, women with a family history of mental retardation and autism were excluded from the current study; therefore, the prevalence of a premutation might be higher without these exclusion criteria. Taken together, women who carry a premutation are at risk for occult POI, with infertility or an elevated FSH and regular or irregular cycles as its only manifestation, as well as overt POI.

The women in this study who carried FMR1 premutations had repeat lengths of 56–91, with only two repeats in the 80–100 range. The relatively low repeat length is surprising given that repeat lengths of 80–100 result in the greatest decrement in average age of menopause and a higher prevalence of overt POI and infertility (Sullivan et al., 2005; Ennis et al., 2006; Allen et al., 2007). On the other hand, a lower repeat range is consistent with the exclusion for a family history suggestive of fragile X spectrum disorders, specifically unexplained mental retardation, fragile X diagnosis and autism. Women with allele sizes between 55 and 70 have a 4–5% chance of expansion to greater than 200 and would be less likely to be excluded from the study based on a suggestive family history.

The relationship between the intermediate repeat allele and overt POI has been controversial (Bretherick et al., 2005; Bodega et al., 2006; Gleicher et al., 2009a; Bennett et al., 2010). The prevalence of the intermediate allele in women with occult POI is higher than in controls in the current data. Further, our study and others demonstrate skewing of the allele distribution curve toward longer allele sizes in women with occult POI, suggesting an association of increased risk of infertility with longer allele sizes (Gleicher et al., 2009a,b). Of note, the definition of premutation and intermediate alleles is based on the likelihood that the repeat will expand to a full mutation to cause fragile X syndrome in progeny, not to describe ovarian function (Nolin et al., 2003). Therefore, a ROC curve was generated to evaluate repeat size as a predictor of ovarian function. The ROC curve area was low and the best cutoff at an allele size of 31.5 resulted in a poor sensitivity and specificity to detect occult POI. Further, a cutoff at the intermediate allele size had a poor positive predictive value for occult POI. Nevertheless, the control group used in the current study is not perfect as it is not known whether control subjects will eventually develop occult POI. Additional studies using a control group with known age at menopause are needed to provide another disease-free population to evaluate the minimum size of the allele predicting ovarian dysfunction.

The ACOG and ACMG guidelines regarding FMR1 premutation testing were put in place by the year 2006, and the ACOG guidelines were revised recently (Sherman et al., 2005; ACOG, 2010). Together, the guidelines recommend testing women with infertility and/or an elevated FSH for the FMR1 premutation based on the phenotype in known carriers (Murray et al., 2000; Hundscheid et al., 2001; Welt et al., 2004; Allen et al., 2007; Rohr et al., 2008). The rate of testing among Boston fertility practices has clearly increased since the guidelines were put in place. Using data from the anonymous samples retrieved through the Crimson pathology service, the authors ‘caught’ all premutations in their infertility practices but missed one intermediate repeat carrier. Thus, the data suggest that testing all women with occult POI can improve the detection of premutation and intermediate alleles, which will identify women in families at risk for fragile X spectrum disorders based on the expected expansion of the repeats across one or two generations (Nolin et al., 2003).

In the current study, genetic counseling was not performed for all women; therefore, the intake family history by the reproductive specialists was used. When compared with the family history taken by genetic counselors, additional history was obtained in 6 subjects, including one family in which a history of unexplained mental retardation in a maternal first cousin was found in a premutation carrier. One of the premutation carriers identified in the current study had a family history of overt POI, which was not used as an exclusion criterion. Taken together, a careful history for fragile X spectrum disorders by the reproductive specialist, including overt POI, identified many of the FMR1 premutation carriers. Nevertheless, problems of recall and incomplete family history information remain, and the majority of the premutation carriers in the current study had no family history. Rajendra et al. (2008) found that half of the newly diagnosed fragile X families are discovered because of the birth of an affected child. Thus, short of population screening, strengthening the current guidelines so that all women with occult POI are tested is necessary to identify more families at risk before having an affected child in the same generation (for premutation carriers) or future generations (for carriers of intermediate alleles).

Although the numbers in the current study were large, the prevalence of the premutation in the controls was low, which could result in a false positive result. The FSH and E2 levels were run in different assays at the participating institutions. Nevertheless, all the institutions used the same reference standard and clinically validated the FSH level.
they used to document occult POI (Nahum et al., 2001; Yanushpolsky et al., 2003; Klipstein et al., 2005). It is possible that additional subjects had occult POI on the basis of an abnormal karyotype. However, we are not able to examine that possibility because a karyotype was not performed in all subjects and we do not have comparison data in the control subjects. Finally, additional causes of infertility were different in the cases than in controls. There were more controls with recurrent miscarriage, uterine anomalies and endometriosis and more women undergoing fertility treatment for lifestyle choice or PGD in the control group and no cases of PCOS or hypothalamic amenorrhea in the cases. These differences reflect definitional issues and the possibility that the control group was more likely to be tested for FMR1 premutations on the basis of these diagnoses.

The FMR1 premutation has been demonstrated to be one of the single largest genetic contributors to age at menopause (Hunter et al., 2008). Data from the current study and others suggest that allele size is longer in women with occult POI and that there is an increased prevalence of the premutation and intermediate alleles in this group as in women with overt POI. Thus, some women with infertility and occult POI will have FXPOI. However, there was no allele size that would serve to detect occult POI with great sensitivity and specificity in the current study. Taken together, the data support recommendations of the American College of Medical Genetics and the American College of Obstetrics and Gynecology for FMR1 repeat testing in women with infertility and elevated FSH levels, strengthening the need for testing in this group of women.

Authors’ roles

C.B.K. and C.K.W. were involved in study design and conception, data collection, analysis, writing, critically revising the manuscript and final approval of the manuscript. V.A.M. and A.C. played a role in study design, data collection, analysis, critically revising the manuscript and final approval of the manuscript.

S.S., J.P., C.R., E.G. and K.L.T. took part in study conception and design, data collection, analysis, writing, critically revising the manuscript and final approval of the manuscript.

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Conflict of interest

A.C. is an employee of Esoterix Genetic Laboratories, LLC, owned by Laboratory Corporation of America Holdings. The company performs DNA testing for fragile X. C.R. is on the board at Origio and received speaker funding from the SMART Conference on behalf of Schering Plough. Both are unrelated to the current manuscript. K.L.T. receives grant support from MERCK-EMD-Serono and Gene Security Network, also unrelated to the current manuscript.

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