

FMR1 Repeats and Ovarian Reserve: CGG Repeat Number does not Influence Antral Follicle Count

Trimble LB Spitzer^{1*}, Erica B. Johnstone², Heather G. Huddleston¹, Marcelle I. Cedars¹, Gina Davis¹ and Victor Y. Fujimoto¹ ¹Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA, USA

²Department of Obstetrics and Gynecology, University of Utah; Salt Lake City, UT, USA

Abstract

Background: Premature ovarian failure has been reported in women carrying FMR1 premutations. This study explored the association between number of FMR1 CGG repeats and Antral Follicle Count (AFC) in infertile women.

Methods: Retrospective cross-sectional study; 350 infertile women underwent FMR1 genotyping and ultrasound for AFC at an academic reproductive medical center. Women with premature ovarian failure were excluded. FMR1 repeats were classified as <45 (normal variation), 45-54 (intermediate allele) or 55-199 (premutation). FMR1 repeats were also classified in groupings to explore subtle differences. The primary study outcome is to assess the effect of FMR1 repeat number on age-adjusted AFC.

Result(s): Only 1 woman carried a premutation, with 57 CGG repeats. Seven women carried intermediate alleles (<2%). FMR1 repeat number was not a significant predictor of AFC in an age-adjusted linear regression when the highest number allele or both allele members were included. Age-adjusted AFC did not differ between FMR1 groups by any of the classification systems used.

Conclusion(s): FMR1 intermediate alleles and premutations are rare in infertile women in the San Francisco Bay Area, with prevalence comparable to the general population. FMR repeat number does not correlate with AFC in this ethnically diverse population.

Keywords: Fragile X; FMR1; CGG repeats; Antral follicle count; Ethnicity

Background

Fragile X syndrome is the most common known hereditary cause of intellectual disability and developmental delay. It results from an expansion of the CGG repeat number in the Fragile-X Mental Retardation 1 (FMR1) gene located on the X chromosome. A full mutation, as is associated with fragile X syndrome, is characterized by 200 or more CGG repeats. With smaller numbers of CGG repeats, fragile X premutations are meiotically unstable and may expand to full repeats within a single or multiple generations [1].

FMR1 premutations, consisting of 55 to 199 repeats, have been associated with Premature Ovarian Insufficiency (POI) [2,3]. Among women who carry an FMR1 premutation, 13-21% has POI compared to only 1% in the general population [4-6].

More controversial is the relationship between FMR1 CGG repeat number of those in a range considered normal and intermediate or "gray zone" alleles, and ovarian function. Sullivan et al. [6] found that the prevalence of POI was 2.2% in carriers of "intermediate alleles" (defined as 41-58 repeats), slightly higher than those women with both alleles \leq 40 repeats (0.9%), but not significantly different. They noted a nonlinear increase in the risk of POI related to the CGG repeat number, with the highest risk (18.6% prevalence) in those with 80-99 CGG repeats [6]. A case-control study suggested that women with POI may be seven times more likely than normal controls to carry intermediate or premutation FMR1 alleles (p=0.04) [7]. A review by Wittenberger et al. [8], also estimated an odds ratio of 2.5 for POI in those with intermediate repeat alleles.

Racial and ethnic differences in FMR1 CGG repeat number have not been thoroughly studied. Recently it was reported that Asians are a more homogeneous group, with CGG count most often ranging between 26-32 repeats, compared to African-Americans or Caucasians, who demonstrated higher proportions outside this range [9].

Previous studies, such as those mentioned above, are heavily

weighted toward women with ovarian failure or incipient ovarian failure. Historically, studies have also included oocyte donors in their study populations [9]. Since these women are preselected based on their ovarian potential, we thought it was important to assess the relationship between FMR1 CGG repeat number and ovarian reserve while excluding both groups, to avoid introducing bias into our results.

In this study, we sought to determine whether the relationship between FMR1 repeat number and ovarian reserve, as assessed by Antral Follicle Count (AFC), would be found in a heterogeneous, multiethnic population of infertile women. We selected AFC as our measure of ovarian reserve as it has been shown to be a better predictor of poor response to ovarian stimulation than age alone or basal FSH; AFC shows a close correlation with the number of oocytes at IVF retrieval [10-14]. A large published meta-analysis comparing AFC with basal FSH confirmed the superiority of the former [15]. Our null hypotheses were that FMR1 repeat numbers do not differ based on ethnicity and that woman with normal or intermediate number alleles do not have increased risk of decreased ovarian reserve, as measured by AFC.

Methods

Patients

We performed a retrospective chart review including infertile women seen in a single university-based fertility clinic between June

*Corresponding author: Trimble Spitzer, MD, UCSF Center for Reproductive Health, 2356 Sutter Street, 7th Floor, San Francisco, CA 94115, USA, Fax: 415-476-6203; E-mail: Trimble.spitzer@ucsfmedctr.org

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2007 to April 2010 who underwent FMR1 genotyping and ultrasound to assess AFC at their initial consultation visit. Only women whose charts contained complete information on AFC, ethnicity, CGG repeat number, age and medical history were included. Women <20 years of age, those with premature ovarian failure and oocyte donors were excluded. A total of 350 women were included in our study. Ethnicities were categorized according to patient self-reporting. Diminished ovarian reserve was defined as an AFC < 5, based on our internal data which shows this threshold to be most specific for predicting poor response to ovarian stimulation, defined by the number of oocytes retrieved. Approval from the UCSF Committee for Human Research/Internal Review Board was obtained prior to conducting this study.

FMR1 analysis

FMR1 testing was completed at different laboratories based on patient insurance coverage or patient preference, In most cases, Polymerase Chain Reaction (PCR) analysis with reflex to Southern blot was used to determine the FMR1 CGG repeat length. While PCR does not reliably detect full mutations, this method allows accurate determination of CGG repeat number for normal, grey zone and premutation alleles. Southern blot reflex allows for exclusion of full mutation status in women with homozygous repeat numbers.

Statistical analysis

We first assessed for ethnic differences in either higher or lower CGG repeat number, using age-adjusted linear regressions. Where differences were found, pairwise comparisons between ethnicities were made. A multivariate linear regression model was then used to assess the effect of FMR1 repeat number on AFC, adjusted for age. In the initial regression, both the higher and lower repeat number alleles were included for each subject. Subsequently, the higher FMR1 CGG repeat number allele was used for each woman. FMR1 repeats were grouped into three previously reported classifications: <45 repeats ("normal variation"), 45-54 repeats ("intermediate allele"), and 55-199 repeats ("premutation") [16].

In efforts to compare our results to previously published data [17], we used 2 additional classification systems. In the first, women were grouped based on both alleles: <28 repeats for either allele; 28-33 repeats for both alleles; \geq 34 repeats for both allele; and one allele <28 repeats with the other >33. In the second, we classified our subjects into 3 groups according to higher CGG repeat number: <35 repeats, 35-50 repeats, and >50 repeats. Comparisons were also made between smaller subdivided groupings to identify any subtle impact that increasing allele repeat numbers may have on AFC.

Repeats (#)	Caucasian (n=190)	East Asian (n=83)	Asian Indian (n=21)	Other (n=52)
<45	184 (96.9%)	83 (100%)	20 (95.2%)	51 (98.1%)
45-54	5 (2.6%)	0	1 (4.8%)	1 (1.9%)
55-199	1 (0.5%)	0	0	0

Table	1:	Demographics	of	race/ethnicity	and	percentage	of	patients	in	FMR1
repeat	nu	mber groups.								

For each of these groupings, we performed a linear regression analysis predicting AFC, adjusting for age. From these models, we generated age-adjusted mean AFCs for each FMR1 allele size, and determined whether this differed from the most common allele pattern.

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Results

There were no full mutations in our study group. One participant carried a premutation, with 57 CGG repeats; only 7 women carried intermediate alleles (2%). None of these patients had a known family history of intellectual disability and only one participant who carried an intermediate allele had a mother who went through premature menopause at age 40. There was no family history of POI in the remaining intermediate allele cohort. Of the 350 women included in our study, 35 were classified as diminished ovarian reserve with an AFC of <5 follicles.

The mean age of women included in the study was 36.0 years (range 20-46) with a mean AFC of 11.7 (range 0-42). Demographics of race/ ethnicity and their distribution among FMR1 repeat classifications are presented in Table 1. 190 women self-identified as Caucasian, 83 women were East Asian and 21 women were Asian Indian. Because the remaining ethnic minorities included very few women in each category, 52 women were collectively categorized as "Other". This category included women of Hispanic, Middle Eastern, African American, and Native American descent. When comparing these 4 ethnic categories, there were no statistical differences in women's age or AFC between groups. There were statistical differences between the lower repeat number of East Asian women compared to Caucasian women, with the mean lower repeat number among East Asians being 2.71 repeats higher than the lower allele of Caucasians (p<0.0005). No statistically significant difference was seen, however, when comparing high CGG repeat number between these racial groups (p=0.3).

FMR1 repeat number was not a significant predictor of AFC in an age-adjusted linear regression when either both allele numbers or only the highest number allele were included. When women were categorized based on the size of both alleles, we found no statistically significant difference in AFC among groups, regardless of number of CGG repeats, when adjusted for age (Table 2). There were also no differences in AFC related to higher allele repeat number when classified as <35, 35-50, or >50 (Table 3) or grouped by 5 CGG repeats (data not shown).

Discussion

Our study identified that FMR1 premutations are quite rare in an ethnically diverse population of infertile women. In our population, after excluding those with POI, we found only 1 premutation in 350 women, a prevalence lower than previously reported [18]. The prevalence of intermediate alleles was 1/52 in our study, similar to the reported national prevalence of 1/57 [19].

There is limited literature on the clinical relevance of the lower CGG repeat alleles, with only one report to date that reported increased risk of DOR with lower repeat numbers [20]. In our study, we found that CGG repeat number was unrelated to AFC across all racial and

Group	N	Mean Age (range)	Mean AFC	Age-Adjusted Mean AFC (for age 36.7)	P-value compared with "normal" (both 28-33)	Difference in AFC from that predicted by age alone
Lower allele CGG repeat number <28	91	36.3 (32-45)	12.8 (0-41)	12.6	0.61	0.16
Both alleles 28-33 repeats	182	36.5 (20-44)	12.3 (0-42)	12.1		-0.33
Higher allele >33	57	37.3 (24-46)	12.9 (0-36)	13.4	0.25	0.93
Lower allele <28 and higher allele >33	18	37.2 (27-39)	10.9 (1-28)	11.3	0.65	-1.18

Table 2: Mean and Age-adjusted mean AFC according to higher and lower FMR1 CGG repeat number.

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Higher FMR CGG Repeat Number	Ν	Mean Age	Mean AFC	Age-Adjusted Mean AFC (for age 36.7)	P-value compared with <35 repeats	Difference in AFC from that predicted by age alone
<35	290	36.5 (20-45)	12.4 (0-42)	12.3		-0.16
35-50	68	37.3 (25-46)	12.6 (0-36)	13.1	0.44	0.60
>50	5	34.8 (24-43)	12 (0-17)	10.6	0.60	-1.8

Table 3: Mean and Age-adjusted mean AFC according to higher FMR1 CGG repeat number groups classified <35, 35-50, >50 CGG repeats.

ethnic groups studied. Since AFC is a marker to reflect potential for successful gonadotropin stimulation, this finding is consistent with the data suggesting that number of oocytes retrieved are similar between all racial and ethnic groups undergoing ovarian stimulation [21-25].

We found that FMR1 CGG repeat number is unrelated to ageadjusted AFC in a large population of infertile women in which those with ovarian failure were excluded. One major difference between our study and those previously published is our exclusion of women with POI. Gleicher et al. [19] published a study of infertile women with >50 CGG repeats with a mean FSH of 38.3 mIU/ml and a standard deviation of 52.1. No information on menstrual histories was provided, suggesting that women meeting criteria for POI were included.

While the relevance of FMR1 testing in women with POI has been established, the utility of this marker in infertile women without the diagnosis of POI, including those with lower than average AFC for age, remains uncertain. Welt and colleagues found increased basal FSH and decreased inhibin B levels in Fragile X premutation carriers compared to age-matched controls with normal ovulatory cycles [26].

In a group of mostly fertile women who were FMR1 premutation carriers undergoing preimplantation genetic diagnosis, a positive correlation was noted between the number of CGG repeats and ovarian response to gonadotropin stimulation with women with CGG repeats in excess of 100 having greater clinical response than those premutation carrier women with less than 100 CGG repeats [27]. While the prevalence of POI among fragile X premutation carriers has been characterized, prevalence ranges of CGG counts in fertile women have not been well established. Thus, it is difficult to relate these findings to any infertile population.

The limitations of our study include the retrospective nature and the relatively small number of premutation and intermediate allele carriers, reflecting the low prevalence in a diverse U.S. population. Secondary to these limitations, an a priori sample size calculation was not performed and a convenience sample was used. Moreover, the sample size may have been inadequate to detect a subtle relationship between FMR1 and AFC. Additionally, we used AFC, not AMH, as an indicator of ovarian reserve. Several studies have correlated sonographic AFC with serum AMH and FSH levels for predicting the ovarian response to gonadotropin stimulation protocols [15,28].

AFC was performed by various clinical staff physicians during the initial patient evaluation. Our internal data support little inter-observer variability in AFC amongst our staff physicians; however, moderate inter-observer variability has been reported in other university-based fertility clinics [29] and must be considered a limitation of this study.

When testing for Fragile X carrier status, PCR analysis with reflex to Southern blot is typically used to determine the FMR1 CGG repeat length. As a rule, PCR does not reliably detect full mutations. However, PCR analysis allows accurate determination of CGG repeat number for normal, grey zone and premutation alleles. Further, Southern blot reflex allows for exclusion of full mutation status in women with homozygous repeat numbers. Due to the clinical nature of our study, FMR1 testing was completed at different laboratories based on patient insurance coverage or patient preference, introducing the possibility of bias in testing methodologies.

Conclusion

Our findings of a low prevalence of FMR1 premutations and gray zone alleles among infertile women, and a lack of relationship between FMR1 repeat number and ovarian reserve, suggest that there may be a threshold effect, over which FMR1 impacts ovarian reserve. Some genetics practices have recommended offering FMR1 screening to all women considering childbearing, while the ASRM has suggested limiting this to women with a family history of Fragile X syndrome, undiagnosed intellectual disability, or personal evidence of POI [30]. Cost-effectiveness analysis has been published in favor of populationbased FMR1 screening [31]. Our findings indicate that infertile women, including those with limited ovarian reserve, do not comprise a high-risk population who should be singled out for FMR1 mutation testing. Given the apparent low prevalence of FMR1 premutations in our population, further confirmation of this preliminary study using a larger sample size appears warranted.

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