Distribution of the *FMR1* gene in females by race/ethnicity: women with diminished ovarian reserve versus women with normal fertility (SWAN study)

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Objective: To study whether reported, but inconsistent, associations between the *FMR1* CGG repeat lengths in the intermediate, high normal, or low normal range differentiate women diagnosed with diminished ovarian reserve (DOR) from population controls and whether associations vary by race/ethnic group.
Design: Case-control study.
Setting: Academic and private fertility clinics.
Patient(s): DOR cases (n = 129; 95 Whites, 22 Asian, 12 other) from five U.S. fertility clinics were clinically diagnosed, with regular menses and no fragile X syndrome family history. Normal fertility controls (n = 803; 386 Whites, 219 African-Americans, 102 Japanese, 96 Chinese) from the United States–based SWAN Study had one or more menstrual period in the 3 months pre-enrollment, one or more pregnancy, no history of infertility or hormone therapy, and menopause ≥46 years. Previously, the SWAN Chinese and Japanese groups had similar *FMR1* CGG repeat lengths, thus they were combined.
Intervention(s): None.
Main Outcome Measure(s): *FMR1* CGG repeat lengths.
Result(s): Median CGG repeats were nearly identical by case/control group. DOR cases had fewer CGG repeats in the shorter *FMR1* allele than controls among Whites, but this was not significant among Asians. White cases had fewer CGG repeats in the shorter allele than Asian cases. No significant differences were found in the high normal/intermediate range between cases and controls or by race/ethnic group within cases in the longer allele.
Conclusion(s): This study refutes prior reports of an association between DOR and high normal/intermediate repeats and confirms an association between DOR and low normal repeats in Whites. (Fertil Steril® 2017;107:205–11. ©2016 by American Society for Reproductive Medicine.)
Key Words: Diminished ovarian reserve, FMR1, race/ethnicity, European Continental Ancestry Group, Asian Continental Ancestry Group, ovarian reserve, infertility, female

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Research has confirmed an association between premutation-level trinucleotide repeat lengths in the *FMR1* gene (55–CGG) and premature ovarian failure in women (also termed primary ovarian insufficiency). The odds ratio for experiencing premature ovarian failure, which clinically presents as a cessation of menses before age 40 and postmenopausal FSH levels, among women who carry the premutation was recently estimated to be 5.4 (95% confidence interval [CI], 1.7–17.4) (1). The association of the *FMR1* gene with other forms of ovarian dysfunction such as pathologic diminished ovarian reserve (DOR) is less clear, as reviewed in 2014 (2). Some reports have suggested that <26 CGG repeat lengths (3), <28 CGG repeat lengths (4), 35–44 CGG repeats (5), 45–54 CGG repeats (6), ≥35 CGG repeats (7), and >40 CGG repeats (8) may be associated with DOR or infertility, while others have reported no association between *FMR1* repeat lengths and DOR (9) or infertility in general (10). The lack of consistency in these infertility reports may be due to analytic differences, such as using alleles rather than women as the unit of analysis (7, 8); having infertility patients as controls (4, 6, 9); and/or not restricting the case definition to DOR (3, 10). It is still an outstanding question whether or not the *FMR1* gene is associated with low ovarian reserve, and if it is, which repeat length confers the greatest risk.

Race/ethnic differences in the *FMR1* CGG repeat distribution have been reported (11–13). Using eight general population studies, Genereux and Laird reported that Asian and non-Asian populations followed similar distributional curves (their analysis was restricted to ≥40 CGG repeat lengths), but the Asian curve was left-shifted and "almost completely non-overlapping" relative to the non-Asian distribution (12). None of the previously cited studies on *FMR1* and DOR examined potential race/ethnic group differences, although race/ethnic variation in the lower allele triplet length has been reported in a cohort of 385 fertility clinic patients unrestricted by cause of infertility (14).

Stratifying the analysis by the higher and lower alleles in females is also important, as some researchers have raised the possibility that the lower allele confers an increased risk of early ovarian aging (4). Only two of the seven *FMR1*/diminished ovarian reserve studies referenced above examined each allele individually (4, 6).

The goal of this study was to determine whether the reported associations between the *FMR1* CGG repeat lengths in the intermediate, high normal, or low normal range discriminate women diagnosed with DOR from women with normal reproductive histories using a general female population comparison group. The analysis investigated each allele individually and examined race/ethnic group differences. The null hypothesis was that the *FMR1* gene distribution below the premutation level (<55 CGGs) would not vary between the women with and without a diagnosis of DOR.

MATERIALS AND METHODS

Case Population Description

Women clinically diagnosed with DOR were enrolled between March 2005 and February 2014 from academic reproductive endocrinology and infertility clinics in California (33% of the participants), North Carolina (19%), and Virginia (15%) as well as from private fertility clinics in Virginia (30%) and North Carolina (3%).

To be eligible as cases, women were required to have a diagnosis of DOR based on elevated but not postmenopausal-level FSH timed to her menstrual cycle; low antimüllerian hormone (AMH) for her age; or fewer than six antral follicles sized 2–10 mm on an ovarian ultrasound (AFC), as detailed elsewhere (5, 15). Additionally, women were required to be ≤42 years old at diagnosis (age requirement was tightened to ages ≤41 in early 2009) and have had regular menstrual cycles for the previous 6 months. Only the Stanford University site, where the high patient volume provided confidence in the consistency of AFC measurement, used the AFC as an entrance criterion. The day 2–5 FSH enrollment criterion was adjusted for the different laboratory machines at each site to ensure consistency in the enrollment criteria across sites, as described elsewhere (5). Approximately 70% of the DOR cases were diagnosed based on elevated FSH, 30% based on low AMH, and 10% based on low AFC, with a subset meeting more than one of those criteria. A woman was excluded as a case if there was a known cause of elevated FSH for her age unrelated to fragile X (e.g., surgical removal of either one or both ovaries, chemotherapy or radiation therapy, Turner syndrome, autoimmune disease) or if she had a family history of fragile X syndrome (FXS) or premutation.

After signing an informed consent, women provided a single blood sample for *FMR1* trinucleotide assessment and received pretest genetic counseling by one of two experienced certified genetic counselors affiliated with the study. Routine demographic information, reproductive history, and family medical history were obtained via self-administered questionnaires and/or review of medical records. This study was approved by the Human Ethics Boards at all academic sites (no. 11448 at the University of Virginia, no. 11-1535 at the University of North Carolina at Chapel Hill, no. 16182 at Stanford University).

Control Population Description

The comparison data are from the Study of Women's Health Across the Nation (SWAN), a multirace, multiethnic, multisite study of the menopausal transition in middle-aged women (www.swanstudy.org). At entry into SWAN, participants were required to be premenopausal, not taking hormones, and between 42 and 52 years of age at the time of enrollment. For further details on the study design, the reader is referred to Sowers et al. (16). From 1996 to 1998, each site recruited a community-based cohort of approximately 450 women. All sites recruited non-Hispanic White women. Additionally, each site recruited women whose self-identified race/ethnic group was African-American (Boston, MA; Detroit area, MI; Pittsburgh, PA; and Chicago, IL), Chinese (Oakland, CA), Japanese (Los Angeles, CA), or Hispanic (Hudson County, NJ).

During years 6 and 7 of the study, materials, including buccal cells and whole blood, were collected from a subset

of participants to provide a source of DNA (17). Enrollees from the New Jersey site did not participate in the SWAN genetics study. DNA samples were available for 1,523 subjects for this analysis (http://datawarehouse.swanrepository.com/aboutInventory.php as of March 6, 2014). This study was approved by the Institutional Review Board at each clinical site, and all women provided written informed consent.

To be eligible for this *FMR1* analysis, women were required to be premenopausal through age 45; have a stored DNA sample; and have undergone menopause at the age $\geq$46. Women could have undergone natural or surgical menopause, provided they were still having periods after the age of 45. This definition has been used elsewhere to define a "normal" age at menopause (1). In addition, women were excluded:

- If they had ever taken fertility medications,
- Ever had a period of 12 months when they could not become pregnant despite regular sexual activity without contraception,
- Had never been pregnant, or
- If the answer to any of these three questions was missing.

There were 805 samples that met these criteria, of which two had insufficient DNA volume, leaving 803 controls available for analysis.

FMR1 Assays

Molecular diagnostic testing on DNA from both cases and controls was performed by the University of Virginia (UVA) Molecular Diagnostics Laboratory using capillary electrophoresis with peripheral venous blood samples on an automated ABI 3700 automated DNA sequencer. The polymerase chain reaction (PCR) contained two primer sets, one flanking the CGG repeat area in the 5′ untranslated region of *FMR1* and an internal control consisting of primers spanning a highly polymorphic region near the promotor of the androgen receptor gene (18). This assay approach yields highly robust counts of the CGG repeat length on the X chromosomes up to 100 repeats and accuracy of plus or minus one CGG repeat (19). DNA samples from controls that displayed only a single peak were presumed to be homozygous, as opposed to having a full mutation on one allele. Cases with single PCR peaks enrolled before April 2012 were confirmed as homozygous with Southern blot testing (62% of cases); cases with single PCR peaks enrolled after that date were presumed to be homozygous. See Pastore et al. (20) for further details on the *FMR1* lab testing.

Statistical Analysis

A power analysis was performed assuming initial estimates of n = 110 cases and n = 680 controls and using published female CGG repeat distributions. The reference distribution was defined as a weighted average of the Streuli et al. (8), Bretherick et al. (21), and Otsuka et al. (22) populations' *FMR1* distributions. These sample size estimates, which were exceeded for this study for both cases and controls, provided 96% power to detect a statistically significant difference (alpha = 0.05) in the underlying CGG repeat distributions.

Because women have two X chromosomes, their *FMR1* results provide two numbers corresponding to the trinucleotide repeat length in each allele. Consistent with prior reports (4, 23, 24), the allele with fewer CGG repeats was termed "allele 1" and the allele with the greater number of CGG repeats was termed "allele 2".

Standard descriptive statistics were calculated for all continuous variables. All FSH values presented have been adjusted to the corresponding cycle day 2–5 value at the primary recruitment site (UVA). No statistical comparisons were performed on the participant characteristics (e.g., demographics) because none of these variables has an influence on the *FMR1* trinucleotide repeat length, with the exception of race/ethnic group, which was controlled in the statistical analysis. Discrete categories for CGG repeat length categories were selected a priori to respond to prior reports of high normal (5, 8) and low normal (3) repeats potentially being associated with early ovarian aging. The categorical distributions of allele 1 and allele 2 CGG repeat lengths were compared across race/ethnic groups using Fisher's exact test with alpha = 0.05. Race/ethnic comparisons were restricted to White versus Asian women, because the sample size of African-Americans in the case group was limited; and data to separate Chinese and Japanese ethnicities were unavailable in the case group. In a previous analysis (20), the SWAN Chinese and Japanese groups had similar *FMR1* CGG repeat lengths. Quantitative comparisons of CGG repeat length, separate for alleles 1 and 2, were completed by one-way analysis of variance, with the allele 2 counts analyzed after log-transformation. A nonparametric Levene's test for homogeneity of the variance across race/ethnic groups was performed using the aggregate alleles.

Secondary analysis compared the proportion of low normal alleles in the DOR cases to the SWAN controls with Fisher's exact tests. Two dichotomous definitions of low normal were used based on the literature ($\leq$25 CGG on both alleles [3] and $\leq$23 CGG on both alleles [25]); This secondary analysis was restricted to White subjects owing to sample size limitations in the other race/ethnic groups. The statistical analysis was conducted using SAS v9.3, and the graphs were created with Stata v13 (StataCorp) and Excel.

RESULTS

Participant characteristics for the two sample populations are displayed in Table 1. As would be expected, the DOR cases had a mean age in the upper 30s (mean = 38 years) and the controls were older than 45 at enrollment into the SWAN study (mean, 47 years). As reflective of the eligibility criteria, few cases had given birth (mean parity = 0.28), while all the controls had been pregnant at least once and the mean parity was 2.29. Corresponding data by race/ethnic group are in Supplemental Table 1. There was no evidence of a difference in the severity of DOR between White and Asian cases (mean FSH and mean AMH were not clinically different between the race/ethnic groups; data not shown).

Graphs of the *FMR1* trinucleotide repeat length stratified by White versus Asian race and by case/control population

TABLE 1

Participant characteristics of the DOR case cohort and SWAN comparison cohort.

Variable	DOR (n = 129)	SWAN (n = 803)
Age (y) at enrollment		
Mean (SD)	37.9 (4.27)	47.2 (2.57)
Median	38.6	47.2
Range	26.6–49.2	42.0–52.9
Marital status (%)		
Single/never married	9 (9.2)	64 (8.2)
Married/partnered	87 (88.8)	544 (70.0)
Separated	0 (0)	25 (3.2)
Widowed	2 (2.0)	24 (3.1)
Divorced	0 (0)	120 (15.4)
Missing (n = 31 cases)		
Ever smoked (%)	18 (14.0)	331 (41.4)
Age (y) at diagnosis of DOR		
Mean (SD)	36.6 (4.0)	NA
Median	37.8	
Range (n)	25.8–42.6	
Race/ethnic group (%)		
White	95 (73.6)	386 (48.1)
African-American	5 (3.9)	219 (27.3)
Asian	22 (17.1)	198 (24.7)
Other	7 (5.4)	0 (0.0)
Parity		
Mean (SD)	0.28 (0.56)	2.29 (1.28)
Median	0.00	2.00
Range	0–2	0–9
Gravidity		
Mean (SD)	1.19 (1.27)	3.09 (1.63)
Median	1.00	3.00
Range	0–5	1–12

Pastore. FMR1 by ethnicity: DOR cases versus controls. Fertil Steril 2016.

are displayed in Figure 1A (allele 1) and 1B (allele 2). For White women, Figure 1A (allele 1) shows that 29 CGG and 30 CGG are the most common trinucleotide repeat lengths among controls and that 28 CGG and 29 CGG were the most common among the DOR cases. A shoulder is apparent around 19–25 CGG in both the case and control groups. For Asian women, more than 60% of the DOR cases had 28 CGG on allele 1, while 29 CGG and 30 CGG were the most common allele 1 repeat lengths among normal fertility controls. Thus, the modal CGG repeat lengths for cases are within one repeat of the modal lengths for controls even after stratification by the two race/ethnic groups. Comparing White versus Asian DOR cases for allele 1, Figure 1A indicates that alleles shorter than 26 CGG were more common in White than Asian women, which is also observed for the SWAN controls, as reported elsewhere (20).

The graphs in Figure 1B (allele 2) for White women overall appear similar between cases and controls. Among Asian women, the graphs show that there are no cases with CGG repeat lengths between 36 and 44, which may be due to the sample size. Comparing the histograms of White and Asian cases, the overall pattern is similar, although the Asian case distribution may have a shoulder around 35 CGG; this observation also holds true for controls. For both cases and controls, note the existence of Whites with fewer than 26 CGG repeats on allele 2 but no Asian women with allele 2 repeat lengths that short.

Summary statistics (mean, SD, median, and range) of the *FMR1* CGG repeat length are shown in Table 2 for all race/ethnic groups. All medians were within one CGG repeat when comparing DOR cases with normal fertility controls, for all races in total and within each race/ethnic group (28 or 29 CGG repeats for allele 1, 29 or 30 CGG repeats for allele 2). The spread of the distribution for allele 1 is greater among White than among Asian women within the DOR cases ($P=.0021$) and within the controls ($P<.0001$); no comparable differences were found for allele 2 ($P=.687$ among cases and $P=.397$ among controls).

The *FMR1* CGG repeat lengths on allele 1, separated by Whites versus Asians and stratified by cases versus controls, are displayed in the top portion of Table 3. There was a significant difference in the CGG repeat lengths of allele 1 between the DOR cases and the SWAN controls among Whites ($P<.0001$) but not among Asians ($P=.239$). This difference was predominantly seen in the proportion of women with very few repeats among the cases, for example, 17.9% of White cases and 3.6% of White controls had fewer than 20 CGG repeats. There was a significant difference in the CGG repeat lengths by race/ethnic group within the DOR cases ($P=.023$) and within the normal fertility controls ($P<.0001$).

The *FMR1* CGG repeat lengths on allele 2, separated by Whites versus Asians and stratified by cases versus controls, are displayed in the bottom half of Table 3. No differences were observed on allele 2 between the DOR cases and the SWAN controls among Whites ($P=.253$) or Asians ($P=.372$). No race/ethnic differences were observed among the DOR cases on allele 2 ($P=.441$). Race/ethnic differences in the CGG repeat length on allele 2 were observed among the controls and have been reported elsewhere (20).

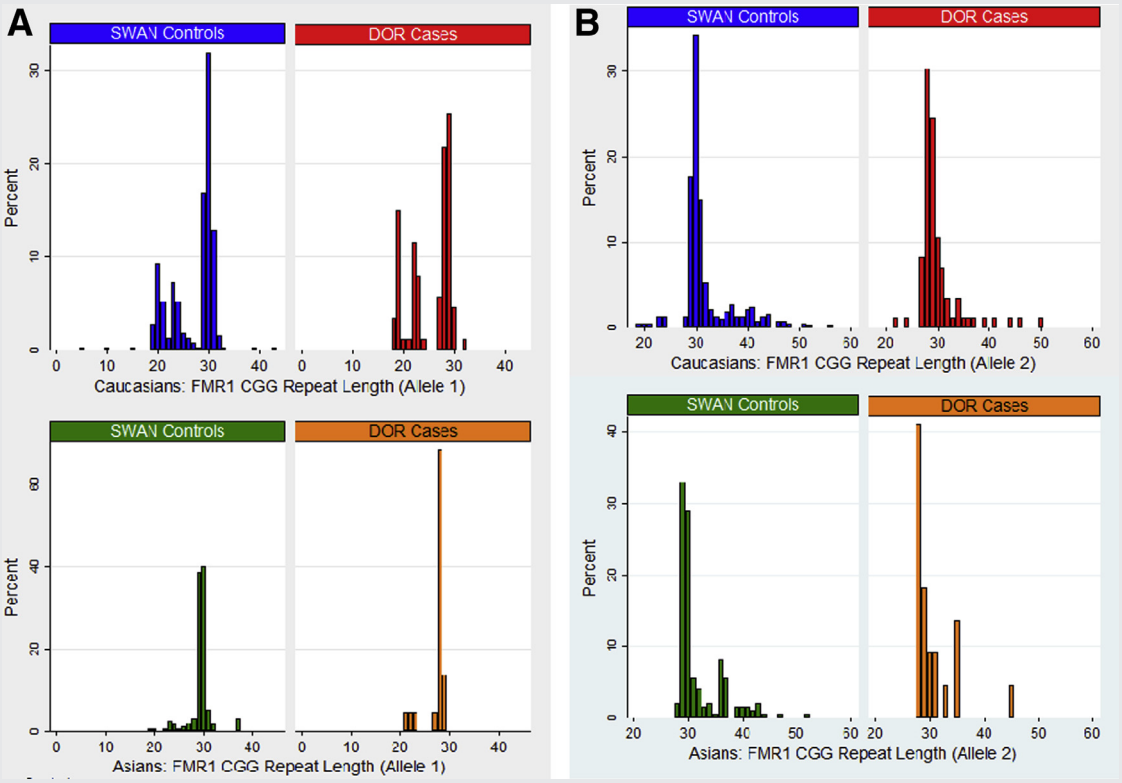
The proportion of cases compared with controls who had both alleles in the "low normal" range (using two definitions from the literature [3, 25]) was not significantly different among Whites in this study. Restricted to Whites, 4.1% (16/386) of the normal fertility controls had $\leq$25 CGG repeats on both alleles versus 3.2% (3/95) of cases ($P=1.00$). Using the second definition of low normal, there were 2.8% (11/386) of the controls with $\leq$23 CGG repeats on both alleles versus 2.1% (2/95) of cases ($P=1.00$).

DISCUSSION

In this study we found significantly fewer CGG trinucleotide repeats in the lower of the two *FMR1* alleles (allele 1) in White women diagnosed with DOR compared with women who have a normal reproductive history. (A larger sample of Asian women may have observed the same finding, but our sample was not large enough to detect this with statistical significance.) White women diagnosed with DOR were also statistically likely to have fewer CGG repeats in the *FMR1* allele 1 compared with Asian DOR cases. No statistically significant differences were found in the CGG repeat length distribution of the higher *FMR1* allele (allele 2) among the DOR cases either in comparison with the SWAN study control population or by race/ethnic group.

The lack of association between a diagnosis of DOR and the CGG repeat length on allele 2 refutes four prior papers

FIGURE 1



(**A**) *FMR1* allele 1 discrete distribution by diminished ovarian reserve cases and normal fertility controls. Allele 1 has the shorter *FMR1* repeat length. (**B**) *FMR1* allele 2 discrete distribution by diminished ovarian reserve cases and normal fertility controls (excluding premutation carriers) Allele 2 has the longer *FMR1* repeat length. The graphs exclude n = 1 White DOR case with 77 CGG repeats.

Pastore. FMR1 by ethnicity: DOR cases versus controls. Fertil Steril 2016.

that reported an association between various CGG repeat lengths and DOR (5–8) but is consistent with two publications (9, 10). Control group definitions are an element that varies across the publications and may contribute to the inconsistent findings. Prior research used the following comparison groups: women referred for genetic testing unrelated to fertility or mental impairment (8), women with no history of infertility (7), women with a natural conception and recent birth (10), and infertility clinic patients with other diagnoses (4, 6, 9). No other reports on this topic had a population-based comparison group as used in this study.

Few reports (only 2) have investigated each individual *FMR1* allele among women with DOR. Lower CGG repeat lengths on allele 1 among women with DOR have been reported by Gleicher et al. (4) but were not observed by Karimov et al. (6) using a case definition that corresponded to a "liberal characterization of diminished ovarian reserve." Neither of those reports analyzed race/ethnic group. The Gleicher/Barad research team has highlighted CGG repeats ≤ 26 as being associated with ovarian reserve in multiple articles including Gleicher et al. (3), based on interquartile ranges, whereas our analysis was not focused on a single dichotomy for "low repeats."

The lack of association between a diagnosis of DOR and having both alleles with few CGG repeats might suggest that low repeat lengths have no true association with early ovarian aging. Thus, even though our data contained a

TABLE 2

Summary statistics for the *FMRI* CGG repeat length by allele, race/ethnic group, and case/control group.

Variable	DOR cases (n = 129): mean (SD), median, range	SWAN controls (n = 803): mean (SD), median, range
Allele 1 CGG repeat length		
Total cohort	25.78 (3.96), 28, 17–35	27.82 (3.95), 29, 5–43
White	25.28 (4.11), 28, 18–32	27.08 (4.46), 29, 5–43
Asian	27.27 (2.21), 28, 21–29	29.23 (2.32), 29, 19–37
African American	26.40 (5.27), 29, 17–29	27.87 (3.82), 29, 8–38
Other	27.29 (4.46), 28, 20–35	NA
Allele 2 CGG repeat length		
Total cohort	30.68 (6.00), 29, 22–77	31.73 (4.75), 30, 19–63
White	30.62 (6.49), 29, 22–77	31.90 (5.12), 30, 19–56
Asian	30.59 (4.04), 29, 28–45	31.89 (4.15), 30, 28–52
African American	29.60 (3.71), 29, 25–35	31.29 (4.57), 30, 20–63
Other	32.57 (6.00), 29, 28–43	NA

Note: Data presented as mean (SD), median, range. Allele 1 has the shorter CGG repeat length, and allele 2 has the longer CGG repeat length. NA = not applicable.

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TABLE 3

***FMR1* CGG repeat length by allele, case/control group, and race/ethnic group.**

	White		Asian	
Variable	DOR (n = 95)	SWAN (n = 386)	DOR (n = 22)	SWAN (n = 198)
Allele 1 CGG repeat length				
<20	17 (17.9)	14 (3.6)	0 (0.0)	1 (0.5)
20–24	22 (23.2)	110 (28.5)	3 (13.6)	11 (5.6)
>24	56 (59.0)	262 (67.9)	19 (86.4)	186 (93.9)
Allele 2 CGG repeat length				
<35	84 (88.4)	313 (81.1)	18 (81.8)	151 (76.3)
35–39	5 (5.3)	31 (8.0)	3 (13.6)	33 (16.7)
40–44	3 (3.2)	30 (7.8)	0 (0.0)	12 (6.1)
45–54	2 (2.1)	11 (2.9)	1 (4.6)	2 (1.0)
>54	1 (1.1)	1 (0.3)	0 (0.0)	0 (0.0)

Note: Data are n (%). Fisher's exact tests for allele 1: case comparison by White versus Asian ($P=.023$); control comparison by White versus Asian ($P<.0001$); White cases versus White controls ($P<.0001$); Asian cases versus Asian controls ($P=.239$). Fisher's exact tests for allele 2: case comparison by White versus Asian ($P=.441$); control comparison by White versus Asian ($P=.013$); White cases versus White controls ($P=.253$); Asian cases versus Asian controls ($P=.372$).

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statistically significant difference in the distribution of allele 1 in White DOR cases compared with in White normal fertility controls, the lack of the association of both alleles having few CGG repeats with DOR shows that the dose-response gradient (26) is not evident in this study population. On the other hand, a dose-response relation may not be biologically relevant for this ovarian phenotype, in which case analysis of a single allele would be more informative than examination of the two alleles simultaneously. Without additional data from other DOR cohorts, this causality question remains unanswered. Scientists with existing *FMR1* data on females with early ovarian aging are encouraged to reexamine their data with a focus on allele 1, to confirm or refute an association between few CGG repeats and low ovarian reserve. Knowing whether low normal repeats have clinical relevance is an important outstanding clinical question, because it is reasonable to extrapolate that *FMR1*-associated infertility would be both inherited and passed to the subsequent generation of daughters. An association between low CGG repeat lengths and reductions in AMH over time have been found in oocyte donors (27), thus providing a possible biologic connection between ovarian reserve markers and *FMR1* outside of DOR studies.

This study found race/ethnic differences in the repeat length distribution in allele 1 but not allele 2 between Asian and White female populations diagnosed with DOR. Race/ethnic variation in the allele 1 triplet length has been reported previously from a large New York City fertility clinic using a cohort of patients unrestricted by cause of infertility (14). In that New York study, Asian female patients, who were predominantly of Chinese heritage, were less likely to have an allele with 25 or fewer CGG repeats than other race/ethnicities ($P<.03$), and no race/ethnic differences were found in the prevalence of repeats >33 CGG. Our findings concur with that publication because our Asian subjects with a diagnosis of DOR were unlikely to have very short CGG repeat lengths.

The primary limitation of this study is the lack of African-American DOR cases, hence we cannot comment on the *FMR1* trinucleotide distribution in that race/ethnic group. Our DOR case sample size is not as large as some prior reports (4, 6, 9) but is larger than in other papers (5, 7, 8). Our comparison population potentially includes women with a family history of FXS because those data were not collected by the SWAN study, but the controls would by definition exclude women with premature ovarian failure. The *FMR1* trinucleotide distributions were combined for the Chinese and Japanese women in this analysis. For the controls alone, the distribution of CGG repeat counts were previously examined and reported separately for each of those Asian groups (20). That previous analysis indicated that the *FMR1* CGG repeat lengths among Chinese and Japanese women are more similar to one another than they are to other race/ethnic groups. Given that the further breakdown of race/ethnicity is not available for the DOR cases, our results are not likely to be biased from the decision to summarize the Asian populations.

The primary strength of this study is having a control population drawn from the general female population rather than infertility clinic patients. Relative to prior research on this topic, having a normal reproductive history in the controls is a strength, as discussed previously. An additional key strength is the ability to examine race/ethnic genetic differences due to the targeted enrichment for race/ethnic groups by the SWAN initial study design. This allowed for analysis of race/ethnic group data separate for alleles 1 and 2 in women diagnosed with DOR, which has not previously been published to our knowledge. The similarity of the proportion of premutation (0.3%) and intermediate (2.3%) length alleles in the controls compared with data in the literature (28, 29) provides support for the adequacy of the comparison population. Our population is well matched to clinical definitions of poor ovarian reserve by requiring regular menstrual periods and has the advantage of representing multiple practices in multiple geographic states. These findings can serve as a reference for researchers.

As people are increasingly screened for genetic conditions, the predictive value of the *FMR1* repeat size will become more significant. Universal *FMR1* screening of pregnant women (30, 31) and preconception patients (30) has been recommended, although not yet adopted. If universal screening is implemented, data from which to interpret those results will be critical. This report does not support universal screening for *FMR1* repeats in populations of women diagnosed with DOR, unless future studies provide evidence of an association between few CGG repeats and DOR. The uncertainty about clinical outcomes with a given size of repeat expansion makes counseling of patients difficult, and an increasing amount of testing by clinicians makes these questions more common. Potential associations between infertility and genes are personally relevant to women in their family planning and for the family planning of future generations, especially in consideration of the trend toward delayed childbearing in women of higher educational levels (32). A clear association between the trinucleotide repeat length and ovarian phenotypes, or a

lack of an association, will need to be demonstrated to allow these individuals, clinicians, and genetic counselors to correctly interpret *FMR1* test results and make informed reproductive decisions. Previous investigators who reported *FMR1* results for only allele 2 are encouraged to reexamine their data on allele 1, to confirm or refute these findings. Consideration of race/ethnic group in future *FMR1* study designs and analyses is warranted based on our study findings.

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SUPPLEMENT TABLE 1

Participant characteristics of the DOR case cohort and SWAN comparison cohort by race/ethnic groups.

	DOR (n = 129)				SWAN (n = 803)			
Variable	**White (n = 95)**	**African American (n = 5)**	**Asian (n = 22)**	**Other (n = 7)**	**White (n = 386)**	**African American (n = 219)**	**Asian (n = 198)**	**Other (n = 0)**
Age (y) at enrollment								
Mean (SD)	37.9 (4.3)	41.6 (2.4)	37.7 (3.20)	36.1 (3.6)	37.9 (4.5)	41.6 (2.4)	37.7 (3.2)	NA
Median	38.6	42.0	37.9	34.7	38.6	42.0	37.9	
Range	26.6–49.2	38.5–44.7	31.4–41.7	31.3–40.9	26.6–49.2	38.5–44.7	31.4–41.7	
Marital status, n (%)								
Single/never married	5 (9.1)	1 (33.3)	0 (0)	0 (0)	28 (7.4)	33 (16.5)	3 (1.5)	NA
Married/living as married	47 (85.5)	2 (66.7)	1 (100)	3 (100)	275 (72.6)	101 (50.5)	167 (84.8)	
Separated	1 (1.8)	0 (0)	0 (0)	0 (0)	8 (2.1)	14 (7.0)	3 (1.5)	
Widowed	2 (3.6)	0 (0)	0 (0)	0 (0)	5 (1.3)	13 (6.5)	6 (3.1)	
Divorced	0 (0)	0 (0)	0 (0)	0 (0)	63 (16.6)	39 (19.5)	18 (9.1)	
Missing (n=31 cases)								
Ever smoked, n (%)	15 (15.8)	1 (20.0)	2 (9.1)	0 (0)	186 (48.2)	103 (47.9)	42 (21.2)	NA
Age (y) at DOR diagnosis								
Mean (SD)	36.63 (4.2)	39.87 (1.7)	36.6 (3.3)	34.3 (3.5)	NA	NA	NA	NA
Median	37.8	39.8	37.2	33.7				
Range	25.8–42.5	37.9–42.6	30.6–41.5	29.6–38.3				
Parity								
Mean (SD)	0.31 (0.58)	0.20 (0.45)	0.23 (0.53)	0.17 (0.41)	2.16 (1.29)	2.71 (1.42)	2.10 (0.96)	NA
Median	0.00	0.00	0.00	0.00	2.00	3.00	2.00	
Range	0–2	0–1	0–2	0–1	0–9	0–9	0–5	
Gravidity								
Mean (SD)	1.07 (1.20)	1.00 (1.00)	1.59 (1.50)	1.43 (1.51)	2.98 (1.66)	3.42 (1.68)	2.95 (1.46)	NA
Median	1.00	1.00	1.00	1.00	3.00	3.00	3.00	
Range	0–5	0–2	0–5	0–4	1–12	1–11	1–11	

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