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