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Prevalence of CGG Expansions of the *FMR1* Gene in a US Population-Based Sample

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Abstract

The primary goal of this study was to calculate the prevalence of the premutation of the *FMR1* gene and of the “gray zone” using a population-based sample of older adults in Wisconsin (n=6,747 samples screened). Compared with past research, prevalence was relatively high (1 in 151 females and 1 in 468 males for the premutation and 1 in 35 females and 1 in 42 males for the gray zone as defined by 45–54 CGG repeats). A secondary study goal was to describe characteristics of individuals found to have the premutation (n = 30, 7 males and 23 females). We found that premutation carriers had a significantly higher rate of divorce than controls, as well as higher rates of symptoms that might be indicative of fragile X-associated tremor ataxia syndrome (FXTAS; numbness, dizziness/faintness) and fragile X primary ovarian insufficiency (FXPOI; age at last menstrual period). Although not statistically significant, premutation carriers were twice as likely to have a child with disability.

Keywords

FMR1 premutation and gray zone CGG expansions; prevalence

INTRODUCTION

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability. FXS is caused by expansion of a trinucleotide (CGG) repeat in the 5′-untranslated region of the fragile X mental retardation 1 (*FMR1*) gene. In individuals with FXS, there are over 200 CGG repeats, compared to the 5–40 repeats in the normal gene. The focus of this study is on the prevalence and associated features of the *premutation*, which is characterized by between 55 and 200 CGG repeats of this gene. We also report the prevalence of the *gray zone*, defined by the American Academy of Medical Genetics as between 45 and 54 repeats [Maddalena et al., 2001] and more recently as between 41 and 54 CGG repeats [Loesch et al., 2007; Hall et al., 2011].

Premutation of the FMR1 Gene

The prevalence of the premutation of *FMR1* is of considerable public health significance because carriers are at greatly increased risk of having a child with the full mutation of FXS. In addition, the premutation itself is associated with a range of emotional and physical problems that become increasingly evident in midlife and old age. According to Hagerman and Hagerman [2002], there is “irrefutable” evidence that some premutation carriers have clinically significant emotional problems, fragile X primary ovarian insufficiency (FXPOI) including early menopause, hot flashes, and infertility, and late-onset Parkinson-like neurological problems (fragile X-associated tremor ataxia syndrome, or FXTAS) including executive function deficits, tremor, ataxia, neuropathy, and brain atrophy. The latter aspects of the phenotype are manifested in late midlife and the early years of old age. None of these problems characterize individuals with FXS, suggesting a unique premutation phenotype [Berry-Kravis et al., 2004]. The prevalence of FXTAS among people over age 50 is estimated at 30% of males and 8–16.5% of females with a premutation [Jacquemont et al., 2004; Pirozzi et al., 2011]. The prevalence of FXPOI is estimated at up to 20% of females with a premutation [Pirozzi et al., 2011].

There is inconsistent evidence regarding comorbid conditions associated with repeat number. Regarding the association between repeat number and cognitive impairments, although Hunter et al. [2008b] did not find elevated rates in premutation carriers, Grigsby et al. [2006] reported a positive association between number of repeats in premutation carrier males with FXTAS and cognitive and functional impairment. Sherman and colleagues noted some evidence supporting the association of repeat number with depression and negative affect in male premutation carriers age 18–50, and with negative affect in females, but no association with anxiety disorders [Hunter et al., 2008a]. Bourgeois et al. [2011] reported significant elevations in rates of mood and anxiety disorders in carriers with FXTAS and to a lesser extent in carriers without FXTAS.

Bailey et al. [2008] also reported significant elevations in a range of psychiatric and behavioral disorders in premutation carriers relative to unaffected samples. Males with the premutation had elevated rates of attention problems, self-injurious behavior, autism, seizures, anxiety, and developmental delay. Females had elevated rates of attention problems, anxiety, depression, and developmental delay. Consistent with Bailey’s findings, two recent studies provided evidence of cognitive impairments in older females [Goodrich-Hunsaker et al., 2011] and males [Cornish et al., 2011] with higher repeats.

The epidemiology and demography of the premutation has only recently begun to be investigated. Population-based prevalence studies have been conducted in Canada, Israel, Spain, Taiwan, and Japan but not in the US. These population-based studies are summarized in Table I, with rates reported separately for males and females. In these studies, prevalence rates varied widely, reflecting significant ethnic variation, likely a function of founder effects. In a review of the literature, Song et al. [2003] pooled estimates across all studies and arrived at a premutation prevalence of 1 in 643 males and 1 in 149 females. This translates into a female to male prevalence ratio of 4.3 to 1, which is considerably higher than the rate predicted by Hagerman [2008] of 2.2 to 1.

A variety of methods were used to arrive at population-based prevalence estimates in research summarized in Table I, including newborn screening, tests of pregnant women, tests of leftover laboratory blood samples, and normal volunteers who responded to a public internet invitation. Apart from universal newborn screening, these methods have significant limitations. The Israeli study [Toledano-Alhadeef et al., 2001] was restricted to pregnant volunteers who self-paid and who did not have a family history of mental retardation. The Japanese study of normal volunteers [Otsuka et al., 2010] included only adults who did not

have major health problems. These factors biased *against* inclusion of premutation carriers. Other studies (not displayed in Table I) have reported prevalence estimates from clinical samples or individuals referred for screening [e.g., Cronister et al., 2008; Hantash et al., 2010]. Although valuable, these studies may be less generalizable to the overall population because they were based on referrals or clinical samples.

Gray Zone Expansions

Past research on gray zone expansions is more limited. The definition of the lower and upper bounds of the gray zone are not firmly established, with some clinical and prevalence studies defining the lower bound at 35 CGG repeats [Rousseau et al., 1995; Bretherick et al., 2005; Curlis et al., 2005] and others defining the upper bound at 60 repeats [Crawford et al., 2001; Curlis et al., 2005; Mitchell et al., 2005]. As noted earlier, the American College of Medical Genetics defines the gray zone as between 45 and 54 repeats, whereas Loesch et al. [2007] suggested that instability begins at 41 repeats. Table II presents a partial listing of gray zone prevalence studies, reflecting significant ethnic variations.

The Present Study

The primary purpose of the present study is to estimate the prevalence of the premutation and gray zone expansions of the *FMR1* gene in a racially and ethnically homogeneous US population. The secondary purpose is to provide preliminary and limited descriptive data on how individuals with premutation expansions differ on selected characteristics from those in the normal range of CGG repeats of the *FMR1* gene. To the best of our knowledge, this is the first population-based US study of the prevalence of the premutation and gray zone expansions, not using clinical samples, to report some data on carriers in early old age, including several symptoms associated with FXTAS and FXPOI.

Based on previous studies of the premutation phenotype [Hagerman and Hagerman, 2002; Sullivan et al., 2005; Bailey et al., 2008; Coffey et al., 2008; Rodriguez-Revena et al., 2009; Chonchaiya et al., 2010], we hypothesized elevated rates in premutation carriers of several symptoms associated with FXTAS (dizziness/faintness, numbness, aching muscles) and FXPOI (age of last menstrual period), as well as depressive symptoms and number of children with disabilities in premutation carriers.

METHODS

Study Population

Data for the present study were obtained from the Wisconsin Longitudinal Study (WLS), a random sample of 10,317 women and men who graduated from Wisconsin high schools in 1957, representing one-third of that age cohort [Hauser et al., 1998]. In 1957, 75% of Wisconsin 18-year olds were high school graduates. Thus, the WLS is representative of that segment of the Wisconsin population who graduated from high school. Follow-up studies were conducted in 1975 with 9,138 (90.1%) surviving members of the original sample when they were, on average, 36-year old; in 1992 with 8,493 (87.2%) of the surviving respondents when they were in their early 50s; and again in 2004 with 7,265 (80.0%) of the surviving respondents when they were in their mid-60s. In addition, parallel data collection procedures were conducted with one randomly selected sibling of a subset of the respondents in 1977, 1994, and 2005, with 5,823 siblings participating in one or more of these data collection points.

Although all of the original WLS participants were high school graduates, as were 93% of their siblings, WLS participants ranged in IQ score from a low of 61 to a high of 145. Fully 15% had IQ scores of 85 (1 SD below the mean) or below. This percentage is nearly the

expected proportion of the population on the low end of the IQ distribution (16% of the population is expected to be 1 SD below the mean or lower). The inclusion of individuals with lower IQs in the WLS population is an important sample characteristic, given past research suggesting a possible cognitive phenotype of the premutation of the *FMR1* gene, as reviewed above. Also important for the present analysis is that the WLS sample is racially and ethnically homogeneous; 99.2% are White and the majority (84.2%) are of Northern European heritage.

In 2006 and 2007, WLS collected saliva samples from both the original graduates and their siblings using Oragene kits (DNA Genotek, Inc., Bethlehem, PA) and a mailback protocol patterned closely on a previous Swedish study [see Rylander-Rudqvist et al., 2006]. Oragene kits were selected because of their ability to be used in a mailback protocol (e.g., no need for immediate freezing) and their high average DNA yield (in our sample, a median 319 $\mu\text{g/ml}$, mean 400 $\mu\text{g/ml}$, and SD of 284 $\mu\text{g/ml}$). All participants provided informed consent under a protocol approved by the Institutional Review Board of the University of Wisconsin-Madison. Compliance with the request to supply saliva was high—56% of WLS survivors from the original respondent and sibling samples ($n = 7,044$). Those who sent saliva had one-half year more schooling (13.9 years vs. 13.4 years, $P < 0.001$), three points higher IQ scores (103.2 vs. 99.5, $P < 0.001$), and higher high school rank (54.6 vs. 46.7, $P < 0.001$) than those who did not return saliva samples. Otherwise, they were representative of the WLS sample as a whole.

The present sample is based on 6,747 cases whose saliva sample yielded sufficient DNA for the CGG repeat assay. Of these, 3,273 (48.5%) were males and 3,474 were females (51.5%). Of the 6,747 cases, 2,632 had a sibling in the analysis (i.e., 1,316 sibling pairs). Siblings' numbers of CGG repeats were correlated ($r = 0.45$). Therefore, to account for the dependence in the sample, the confidence intervals for the prevalence estimates were calculated using a bootstrapped sampling approach. This approach viewed the participants as nested within "families" (where siblings are members of the same family). For females and males separately, we generated random samples (with replacement) of N families, where N is equivalent to the number of unique families in each sex-specific sample. If a family with same-sex siblings was selected, both siblings were included in the bootstrap sample. We created prevalence estimates from 10,000 of these samples, and the 2.5 and 97.5 percentiles of the bootstrapped sample distributions were reported as the 95% confidence intervals. The bootstrap procedure was performed in R, adapted from example code provided by Good [2006], which is available from the authors upon request.

Determination of the *FMR1* CGG Triplet Repeat Number

The number of *FMR1* CGG repeats was determined for all samples using a PCR-based protocol that incorporated reagents developed and manufactured by Celera Corporation (Alameda, CA). These reagents are commercially available through Abbott Molecular (Des Plaines, IL). The protocol combined gene-specific primers that flank the CGG repeat region of the *FMR1* gene with gender-specific primers, a polymerase mixture, and a reaction buffer that is optimized for amplification of GC-rich DNA. The PCR reactions were carried out in 96-well plates, and each well contained a 20 μl reaction volume that consisted of 13 μl of High GC PCR buffer, 0.8 μl of *FMR1* primers, 0.6 μl of gender primers, 1.2 μl of TR PCR Enzyme Mix, 1.4 μl of water, and 3 μl of 5–10 ng/ μl DNA template. All reagents and the reaction plate were placed on ice throughout the duration of assay setting-up.

PCR was performed on a ABI Veriti thermal cycler (Applied Biosystems, Grand Island, NY) using two sets of cycling parameters that were automatically implemented serially by the system. Conditions for the first set of 15 cycles were: 98.5 °C for 10 sec, 58 °C for 60

sec, and 75 °C for 6 min. Conditions for the second set of 15 cycles were: 98.5 °C with 0.1 °C increment per cycle for 10 sec, 56 °C for 60 sec, and 75 °C for 6 min.

Upon completion of PCR, 3 µl of CleanUp Enzyme Mix was added to 2 µl of PCR product to reduce the stutter ($n - 1$) signal typically observed with the amplification of GC-rich DNA targets. The mixture was incubated at 75 °C for 10 min followed by the addition of 17 µl of Hi-Di™ Formamide (Applied Biosystems) and 3 µl of ROX™ 1000 Size Standard (Celera Corporation). The function of ROX™ 1000 Size Standard was to accurately size all PCR products between 75 and 1,000 bp. This size standard consists of 20 single-stranded DNA fragments of known sizes each labeled with X-Rhodamine (ROX). The DNA fragment sizes are as follows: 50, 75, 100, 200, 300, 350, 400, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, and 1,000 bp. Samples were subsequently denatured at 93 °C for 30 sec before undergoing electrophoresis on an ABI 3730xl with POP-7® polymer using a 50-cm array. The sample was injected twice in order to accommodate optimal capillary electrophoresis conditions for a wide range of CGG repeats. ABI 3730xl run module settings are similar to those previously described [Lyon et al., 2010]; the major differences are Injection Time (1 sec in injection 1 and 22 sec in injection 2 vs. 8 sec) and Run Time (4,000 sec in injection 1 and 5,500 sec in injection 2 vs. 2,000 sec). To ensure the consistency of assay performance, in each PCR/CE run, a size control DNA sample was also processed along with the testing samples. The size control samples used in this study are Coriell NA06891, NA06910, and NA20239.

Data from each run were analyzed using GeneMapper® v. 4.0 (Applied Biosystems). CGG triplet repeats were calculated using the following formula: number of CGG repeats = (peak size – 193)/3.

The protocol also detects the presence of X and Y chromosomes within a sample. This information enabled sex confirmation and helped identify female samples with a single detectable CGG repeat (apparent homozygosity). Those samples were further evaluated by agarose gel electrophoresis for the presence of large full mutation alleles. A genomic Coriell DNA sample with 645 CGG triplet repeat (NA04025) was incorporated in each PCR plate, and verified on agarose gel electrophoresis.

Measurement of Phenotypic Characteristics

Two rounds of data from the WLS were used to measure the variables hypothesized to be characteristic of the premutation phenotype: data collected in 1992/94, when respondents averaged 53–54 years of age, and in 2004/05, when they averaged 65–66 years of age. At both rounds of data collection, respondents were queried about their health, mental health, and demographic characteristics via telephone interviews and mailback questionnaires.

In 2004/05, respondents were asked to self-report whether they had any of a list of symptoms, three of which were associated in past research with FXTAS: dizziness/faintness [Chonchaiya et al., 2010], numbness [Coffey et al., 2008], and aching muscles [Coffey et al., 2008; Rodriguez-Revena et al., 2009]. The frequency of these symptoms was rated as weekly or more often (1) versus less often than weekly (0). Women were asked to report age at last menstrual period (measured in 2004/05). Women were also asked whether their ovaries or uterus had been surgically removed, and those who answered affirmatively were not included in the analysis of age at last menstrual period.

Depressive symptoms were assessed by the Center for Epidemiological Studies-Depression Scale (CES-D) [Radloff, 1977]. For each of 20 depression symptoms, the respondent was asked to indicate how many days in the past week the symptom was experienced (0 = never to 3 = 5–7 days; $\alpha = 0.85$ for both the 1992/94 and 2004/05 rounds of data collection). The

total score is the sum of the category ratings for the 20 items. Finally, respondents were asked whether any of their children had a developmental disability or a serious mental health problem, and if so, what condition.

Statistical Analysis of Phenotypic Characteristics

In order to determine whether individuals with the premutation who were identified in the WLS differed from the overall WLS population with respect to select symptoms associated in past research with FXTAS, FXPOI, depressive symptoms, and the likelihood of having a child with a disability, we constructed a comparison group of WLS respondents with CGG repeats in the normal range (<41 CGG repeats), stratified by gender to match the male-to-female ratio in the premutation sub-group. (We included the greatest possible number of WLS controls to match the gender ratio in the premutation group.) The comparison between the premutation group and the comparison group was conducted using ANCOVA with age as a covariate.

RESULTS

Prevalence of Premutation and Gray Zone Expansions

A total of 6,747 samples were assayed for CGG repeat number, which ranged from 9 to 135. The modal repeat number was 31, characteristic of 38.1% of the sample. There were 30 cases in the premutation range. We identified 176 individuals with gray zone expansions when the gray zone is defined as between 45 and 54, and 429 cases in the gray zone as defined as between 41 and 54 repeats (Fig. 1 and Table III).

The premutation prevalence was 1 in 151 women (95% boot-strapped confidence interval [CI]: 1 in 249 to 1 in 105) and 1 in 468 men (95% CI: 1 in 1,628 to 1 in 252). The female to male prevalence ratio was 3.1 to 1 (95% binomial CI: 1.3–7.2).

Using the definition of the gray zone of between 45 and 54 repeats, we found that 1 in 35 women had a repeat number in the gray zone (95% CI: 1 in 44 to 1 in 29), compared to 1 in 42 men (95% CI: 1 in 54 to 1 in 34), a ratio of 1.2 females to 1 male (95% binomial CI: 0.9–1.6). Using the definition of the gray zone between 41 and 54 repeats, we found that 1 in 13 women had a repeat number in the gray zone (95% CI: 1 in 15 to 1 in 11), compared to 1 in 21 men (95% CI: 1 in 25 to 1 in 18), a ratio of 1.6 females to 1 male (95% binomial CI: 1.3–1.9).

Characteristics of Individuals With the Premutation

Background and demographic characteristics—We checked the comparability of those with the premutation and the control group with respect to family background when they averaged 18 years of age in 1957 (the first year the WLS collected data), as well as their demographic characteristics in midlife (age 53–54) and the early years of old age (age 64–65). The premutation carrier sample members were 2 years older, on average, than the comparison group, and thus in all other comparisons age was covaried. There was no difference in family income in 1957 or high school IQ score (the premutation carriers averaged an IQ of 105 and the comparison group averaged 104). The two groups ultimately attained an average of nearly 14 years of education, not a significant difference.

By midlife (age 53–54), the two groups remained similar in most demographic characteristics, including employment status (over 80% employed either full or part time) and number of children (an average of almost 3). Unexpectedly, the premutation carriers were significantly more likely to be divorced in their early 50s (26.7% divorced at age 53–54 vs. 10.4% in the comparison group, chi square = 8.26, $P < 0.01$). The greater likelihood

of being divorced was also evident in the early years of old age (21.4% divorced at age 64–65 vs. 10.0%, chi square = 4.02, $P < 0.05$). This difference in the probability of being divorced was not due to having a child with a disability, as those who were not divorced (i.e., those who were married or widowed) had a higher likelihood of having a child with a disability than those who were divorced, although this was not a statistically significant difference (27.3% of premutation cases who were married or widowed had a child with a disability vs. 12.5% for divorced premutation cases). In other respects, the premutation carrier and control groups were comparable (e.g., employment/ retirement rates and whether they had adult children living at home).

Hypothesized differences—As shown in Table IV, focusing on the women only, the two groups differed significantly with respect to the exact age of their last period (48.1 years of age for the premutation group vs. 50.8 for the controls, $P < 0.05$).

Regarding the hypothesized differences in symptoms associated with FXTAS, when the men and women in this sample were in their mid-60s, there were no group differences in self-reports of aching muscles, but there was a significantly higher rate of dizziness/ faintness (17.9% weekly or more often for the premutation group vs. 3.9% for the controls, $P < 0.001$) and numbness (28.6% weekly or more often for the premutation group vs. 13.3% for the controls, $P < 0.05$). Counter to our hypothesis, there was no difference between the premutation carriers and controls in depression score either in their early 50s or mid-60s.

Finally, nearly a quarter of premutation carrier parents had a child with a disability (23.3%) as compared with 11.9% of parents who had normal repeat lengths, a difference that approached statistical significance ($P = 0.07$). For descriptive purposes, of the seven premutation carrier parents who had a child with a disability, two had children with intellectual disabilities, one had a child with a learning disability, and four had a child with mental health problems—one with major depression, two with bipolar disorder, and one with alcohol and drug problems.

DISCUSSION

To the best of our knowledge, this is the first population-level non-clinical US study of the prevalence of premutation and gray zone expansions, and the second-largest population-based study in the world literature of the prevalence of *FMR1* expansions to include both males and females. In our sample, we observed a premutation prevalence rate of 1 in every 468 males, which is the second highest population prevalence rate for males reported in the literature (second to Fernandez-Carvajal et al. [2009], which was a Spanish newborn screening study) and a premutation prevalence rate of 1 in every 151 females, which is the second highest population prevalence rate reported for females in the literature (second to Toledano-Alhadeff et al. [2001], which was an Israeli study of pregnant women with no history of developmental disabilities). Female premutation prevalence was nearly the same in a sample of women referred for cystic fibrosis screening, which reported a premutation prevalence of 1 in 178 when the authors adjusted their sample to match the racial/ethnic composition of the US [Hantash et al., 2010].

The present sample consisted of Whites of primarily Northern European descent, a group not yet studied in population-based *FMR1* prevalence research, but one where the prevalence appears to be relatively high. As the prevalence was calculated using population screening, rather than being ascertained via affected family members, there were larger numbers of carriers with fewer repeat lengths (i.e., closer to 55) which is another reason for the higher prevalence rate. However, the premutation prevalence of the present study is lower than the prevalence found in some clinically referred samples, one of which reported

the premutation to occur in 1 in 71 females [Cronister et al., 2008]. In another study, the prevalence of the premutation was lower than the present study at 1 in 382 among women with no known or suspected family history of fragile X and who consented to fragile X screening [Cronister et al., 2005].

Past research has shown that *FMR1* expansions vary considerably across ethnic groups, suggesting significant founder effects. Because most past research has consisted of single-sex and single-ethnicity studies, pooling data across studies may give misleading estimates. This may be a particularly important problem in estimating sex ratios of the prevalence of premutation expansions. The pooled estimates by Song et al. [2003] describe a female to male premutation prevalence ratio of about 4.3 to 1, which is considerably higher than the 2.2 to 1 ratio predicted by Hagerman [2008]. Inspection of Table I suggests that pooling estimates from single-sex studies across different populations may confound the observed female to male prevalence ratio. For example, in the studies summarized by Song et al., (2003), the overall premutation sex ratio was 2.6 to 1 among studies that simultaneously measured females and males, whereas it was 4.1 to 1 when pooling the studies that included either only males or only females. Further, the female premutation prevalence in female-only studies was roughly double the female premutation prevalence in studies that also included males, suggesting that the studies that included males might have been conducted primarily in populations with a lower overall premutation prevalence. In the present study, we observed a female to male prevalence ratio of 3.1 to 1, but the confidence intervals of this estimate include the expected 2.2 to 1 ratio predicted by Hagerman [2008]. Large population-based studies that simultaneously include males and females are needed to determine whether the sex ratio for the *FMR1* premutation truly exceeds 2.2 and the potential mechanisms underlying this discrepancy.

Comparison of the prevalence of gray zone expansions in the present study with past reports is complicated because of inconsistencies in defining the gray zone. We decided to report the gray zone prevalence using two accepted definitions in order to provide the most complete information. However, differences in the definition of the gray zone from study to study as well as ethnic variation complicate the comparison of prevalence estimates.

The world literature contains surprisingly few population-level studies of the prevalence of *FMR1* expansions. One possible reason is the need for high throughput approaches. In past research, the capillary-electrophoresis-based *FMR1* CGG repeat assay has been reported in both a qualitative and quantitative manner. The qualitative assay classifies samples into three groups: no expansion, premutation, or full mutation [Hantash et al., 2010; Lyon et al., 2010] and does not permit exact sizing. The quantitative assay provides the exact number of CGG repeats [Larsen et al., 1997]; however, the quantitative assessment reported in that study was limited to a capacity of 48 samples per run. In the present study, CGG repeat number was assayed using a higher throughput platform processing 96 samples per run, which might make future large population screening studies more feasible. In this respect, the present study is an advance.

Although estimating the prevalence of premutation and gray zone expansions in a US population was the primary goal of the present study, a secondary purpose was to provide descriptive data about phenotypic characteristics of identified cases. We did so by capitalizing on the existing survey data of the WLS, which was collected over the past 50 years to characterize broadly defined life course trajectories in demographic, social, and health characteristics. The WLS is not a clinical study, and thus is limited in the clinical sensitivity of phenotypic data, but it does offer two unique characteristics. First, all previous studies of the phenotype of the premutation of *FMR1* are based on samples identified through a full-mutation family member. In contrast, the WLS premutation cases were

ascertained via population screening. It can be assumed that most sample members were unaware that they had a *FMRI* expansion. Thus, their self-reports may be less biased than reports of symptoms in clinical samples. Second, our study had a larger proportion of carriers with low premutation repeats than previous studies describing the premutation phenotype; fully 14 individuals (46.6%) had repeats between 55 and 60. In future studies, it will be important to extend examination of the lower bound of premutation repeats, which possibly are associated with a less severe phenotype.

Nevertheless, some evidence of phenotypic differences was observed. Specifically, women with the premutation reported their last menstrual period to be nearly 3 years earlier than controls (48.1 years vs. 50.8 years of age), consistent with past clinical research [Pirozzi et al., 2011]. There was also suggestion of FXTAS-like symptoms in carriers in the present sample. Past research also has shown that approximately 30% of premutation male carriers age 50 and older and about half of that number of female carriers have clinically defined FXTAS [Pirozzi et al., 2011]. In our sample, that would predict 5 or 6 affected carriers, but 11 members of our sample reported FXTAS-like symptoms (either dizziness/faintness, numbness, or both), significantly higher than the controls. Currently ongoing data collection from the WLS sample members at age 71–72, including in-person assessments of physical and cognitive functioning, may make it possible to both deepen this phenotypic description and extend it almost decade later in the life course.

It was also notable that premutation carriers did not differ from controls in their level of depression, and were similar in many other respects (education, employment, retirement). Yet they were considerably more likely to be divorced at both assessment points (i.e., early 50s and mid-60s). Most past studies of premutation carriers reported the same rates of divorce in carriers and controls [e.g., Kogan et al., 2008; Roberts et al., 2009], but these carriers were research volunteers who were ascertained after a child with full mutation FXS was diagnosed in the family, so it is possible that such volunteers were different in marital functioning than our population-based sample.

This study was not without limitations. Although larger than many previous studies of the prevalence of the premutation, the present study was small in size and limited in statistical power particularly in the description of phenotypic characteristics. The small number of premutation carriers made it impossible to test for differences separately for males and females, both due to constraints on statistical power and also in line with IRB restrictions on analyses that might lead to the identification of individuals. It should be further emphasized that the measures of the characteristics of the sample members with and without expansions were obtained through a survey with data supplied by respondents; no independent clinical assessment of the veracity of their self-reports was available. In addition, although a strength of the study is the homogeneity of the sample in race and ethnicity, the prevalence rates should not be generalized beyond populations of Northern European descent. Although the sample was about average in IQ score (mean = 105), almost all were high school graduates. The WLS is not representative of the one-quarter of the Wisconsin population who were high school dropouts during the middle years of the last century, nor of those who died before their mid-60s when saliva was collected. Thus, the reported prevalence might underestimate true prevalence.

To conclude, we found that expansions of the *FMRI* gene are more prevalent in a sample of Northern European descent than in many other racial and ethnic groups. As reported in previous clinical studies, such expansions appear to confer risk for carriers, beyond having a child with FXS. Yet there are many unanswered questions. Longitudinal study of populations, such as the WLS, where repeat size has been ascertained, would permit intra-individual descriptions of phenotypic manifestation of the premutation across the years of

adulthood and old age, and multigenerational family studies would provide insights into population-level patterns of transmission of CGG repeats across generations.

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FIG. 1. Frequencies of CGG repeats: gray zone and premutation (n = 6,747)

TABLE I

Published Studies of the Prevalence of the Premutation*

Refs.	Prevalence	# Premutations detected	Sample size	Location	Type of study
Males					
Fernandez-Carvajal et al. [2009]	1 in 251	21	5,267	Spain	Newborn screening
Dombrowski et al. [2002]	1 in 813	13	10,572	French Canadian	Blood samples
Rife et al. [2003]	1 in 1,233	4	5,000	Spain	Newborn screening
Tzeng et al. [2005]	1 in 1,674	6	10,046	Taiwan	Newborn screening
Otsuka et al. [2010]	—	0	513	Japan	Healthy volunteers
Song et al. [2003]	1 in 643	31	19,929	—	Literature review
Females					
Toledano-Alhadeef et al. [2001]	1 in 113	113	14,334	Israel	Pregnant women with no family history of MR
Berkenstadt et al. [2007]	1 in 157	255	40,079	Israel	Pregnant women with no family history of MR
Rousseau et al. [1995]	1 in 259	41	10,624	French Canadian	Blood samples
Otsuka et al. [2010]	—	0	324	Japan	Healthy volunteers
Song et al. [2003]	1 in 149	321	47,712	—	Literature review

*The premutation ranged from 55 to 200 repeats.

TABLE II

Published Studies of the Prevalence of the Gray Zone

Refs.	Prevalence	# Gray zone cases detected	Sample size	Location	Type of study and definition of the gray zone
Males					
Fernandez-Carvajal et al. [2009]	1 in 26	199	5,267	Spain	Newborn screening, 45–54 repeats
Dombrowski et al. [2002]	1 in 294	36	10,572	French Canadian	Blood samples, 34–53 repeats, but concerns about sensitivity for medium-sized alleles
Tzeng et al. [2005]	1 in 1,674	6	10,046	Taiwan	Newborn screening, 45–54 repeats
Otsuka et al. [2010]	1 in 103	5	513	Japan	Healthy volunteers, 40–50 repeats
Pefiagarikano et al. [2004]	1 in 32	5	158	Basque	Healthy unrelated males, 40–54 repeats
Females					
Rousseau et al. [1995]	1 in 128	83	10,624	French Canadian	Blood samples, 34–53 repeats, but concerns about sensitivity for medium-sized alleles
Otsuka et al. [2010]	1 in 324	1	324	Japan	Healthy volunteers, 40–50 repeats

TABLE III
Individuals With Premutation and Gray Zone Expansions in the Wisconsin Longitudinal Study

	Males	Females	Total
<45 CGG repeats, n (%)	3,188 (97.4)	3,353 (96.5)	6,541 (97.0)
45–54 CGG repeats, n (%) ^a	78 (2.4)	98 (2.8)	176 (2.6)
55–200 CGG repeats, n (%)	7 (0.2)	23 (0.7)	30 (0.4)
Total, N (%)	3,273 (100)	3,474 (100)	6,747 (100)

^aThe number of males and females who have between 41 and 44 repeats were 79 and 174, respectively. Thus, the gray zone as defined between 41 and 54 repeats [Loesch et al., 2007] would total 157 males and 272 females.

TABLE IV
Phenotypic Characteristics Hypothesized to Differ in Premutation Carriers Versus Normal Controls

Female only analysis	Premutation (n=20 females)	Normal controls (n = 1,893 females)	Statistic^a
Age at last menstrual period	48.1	50.8	F=4.22, <i>P</i> < 0.05
Whole sample analysis	Premutation (n=30 males and females)	Normal controls (n = 2,800 males and females)	Statistic
Aching muscles ^b	0.43	0.43	F = 0.00, ns
Dizziness/faintness ^b	0.18	0.04	F=13.93, <i>P</i> < 0.001
Numbness ^b	0.29	0.13	F=5.28, <i>P</i> < 0.05
CES-D–1992/94	8.72	8.69	F = 0.02, ns
CES-D–2004/06	5.76	7.29	F = 1.09, ns
Has child with a disability	0.23	0.12	F=3.41, <i>P</i> = 0.07

^aNote: F-tests were adjusted for age difference between premutation carriers and normal controls. Unadjusted means are reported.

^bAching muscles, dizziness/faintness, and numbness are reported for 2004/05 (age 64–65) and coded 0 = monthly or less often, 1 = weekly or more often.