Psychiatric Disorders Among Women With the Fragile X Premutation Without Children Affected by Fragile X Syndrome

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Several studies have demonstrated increased rates of anxiety and depressive disorders among female carriers of the fragile X premutation. However, the majority of these studies focused on mothers of children with fragile X syndrome, who experience higher rates of parenting stress that may contribute to the emergence of these disorders. The present study compared psychiatric symptom presentation (utilizing measures of current symptoms and lifetime DSM-IV Axis I disorders) in 24 female carriers without affected children (mean age = 32.1 years) to 26 non-carrier women from the community (mean age = 30.5 years). We also examined the association between CGG repeat size (adjusted for X activation ratio) and mRNA, with severity of psychiatric symptoms. Women with the premutation reported significantly elevated symptoms of anxiety, depression, interpersonal sensitivity, obsessive-compulsiveness, and somatization relative to controls during the past week. Carriers had significantly higher rates of lifetime social phobia (42.3%) compared to controls (12.5%); however, this comparison did not remain significant after multiple comparison adjustment. Rates of other psychiatric disorders were not significantly elevated relative to controls, though it should be noted that lifetime rates among controls were much higher than previously published population estimates. Although the sample is relatively small, the study of this unique cohort suggests the premutation confers risk for mood and anxiety disorders independent of the stress of parenting children with FXS. Screening for psychiatric disorders in women with the premutation, even before they become parents, is important and highly encouraged. © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

Fragile X syndrome (FXS) is the most common hereditary cause of intellectual disabilities (ID) and the most common single gene cause of autism [Turner et al., 1996; de Vries et al., 1998; Kooy et al., 2000]. This disorder is caused by disruption in the expression of the fragile X mental retardation (FMR1) gene, found on the long arm of the X-chromosome [Verkerk et al., 1991]. This region includes an abnormal trinucleotide CGG repeat expansion of 55–200 repeats in premutation carriers, and over 200 repeats in individuals with the full mutation and FXS. While the prevalence of the full mutation, typically leading to FXS, is relatively low, affecting approximately one in 4,000–5,000 individuals [Coffee et al., 2009], the premutation is much more common, occurring in up to one in 250 females and one in 250–800 males, depending on regional differences [Rousseau et al., 1995; Dombrowski et al., 2002; Hagerman, 2008; Fernandez-Carvajal et al., 2009; Tassone et al., 2012; Maenner et al., 2013]. In contrast to the full mutation, the premutation typically does not cause a substantial reduction in FMRP levels, but instead leads to increased production of FMR1 mRNA, correlated with CGG repeat length (2–8 times normal levels) [Tassone et al., 2000b]. Female premutation carriers typically have normal development and intellectual function, but are at increased risk for developing primary ovarian insufficiency [FXPOI; Wittenberger et al., 2007], depression and anxiety [Johnston et al., 2001; Hessl et al., 2005; Bailey et al., 2008; Roberts et al., 2009; Adams et al., 2010; Bourgeois et al., 2011; Cordeiro et al., 2015], hypothyroidism, and late-onset neurological problems such as neuropathy, fibromyalgia, and the fragile X-associated tremor ataxia syndrome (FXTAS) [Bourgeois et al., 2007; Coffey et al., 2008; Liu et al., 2013; Wheeler et al., 2014]. Because the premutation is relatively common in the general population, practicing mental health practitioners will very likely encounter these individuals.

FXTAS and FXPOI are the most well-known and well-defined disorders in premutation carriers [Sullivan et al., 2005; Hagerman and Hagerman, 2013]. Although mounting evidence suggests a heightened risk for some psychiatric disorders, especially mood and anxiety disorders, in carriers, most studies relied primarily on individuals with FXTAS or mothers of children with FXS [Roberts et al., 2009; Bourgeois et al., 2011]. Because parenting children affected by developmental disability and/or autism is associated with increased rates of emotional stress and health [e.g., Miodrag and Hodapp, 2010], the studies make it difficult to determine whether the premutation itself is a risk factor, independent of the indirect effects of these other major life stressors. An exception was Franke et al. [1998] who carried out a study to determine whether psychological problems were related to the mutation itself or to the stress of raising a developmentally impaired child. This study compared 13 mothers with the full mutation, 61 mothers with the premutation, 17 women with the premutation who were siblings of the first two groups but did not have children with FXS, 18 women siblings without the FMR1 mutation and without children, and 42 mothers without the FMR1 mutation who had children with autism. Mothers with a premutation, as well as their siblings without affected children, were more likely to be diagnosed with social phobia than a control group of mothers of children with autism. Also, Roberts et al. [2009] reported that mothers of affected children with a history of major depression retrospectively reported that their illness occurred before the birth of their child, providing preliminary evidence of a more direct effect of the FMR1 premutation as a risk factor.

The findings of this prior work suggest that the premutation itself contributes to anxiety and its sequelae and even in childhood anxiety can be associated with the premutation [Cordeiro et al., 2015]. However, this conclusion has been difficult to confirm because the majority of women participating in the aforementioned studies were mothers of one or more children diagnosed with FXS. The number of children with FXS is associated with the likelihood of developing postpartum depression in mothers with the premutation, with a 158% increased risk with each additional affected child [Obadia et al., 2013]. In addition to the psychological impact of raising affected children, these mothers are faced with significant parenting stresses, child behavioral challenges, and associated changes in the dynamics of the home and family environment [Hessl et al., 2001; Johnston et al., 2003]. Studies have shown a strong relationship between the behavioral presentation of the child with FXS and associated changes in maternal stress levels [Johnston et al., 2003; Wheeler et al., 2007]. The level of child problematic behavior is significantly related to maternal stress level, and likely exacerbates maternal symptoms of depression and anxiety [Wheeler et al., 2007]. These stressors are known to impact the central stress response system, the hypothalamic–pituitary–adrenal axis (HPA axis), detrimentally in mothers of children with FXS [Seltzer et al., 2012], a phenomenon that appears to be dependent on the premutation allele size. In general, it is widely understood that mothers raising children with disabilities have higher levels of depression, stress, and anxiety compared to women from the general population [Dumas et al., 1991; Blacher et al., 1997; Hoare et al., 1998; Veisson, 1999; Olsson and Hwang, 2001; Baker et al., 2002; Glidden & Schoolcraft, 2003; Hastings, 2003; Saloviita et al., 2003].

In this study, we aimed to fill a gap in the knowledge of psychological health of women with the premutation by carefully evaluating a cohort that does not have children affected by FXS or any disability. Based on previous literature and clinical experience we expected that, compared to controls, women in the premutation group would report higher levels of psychiatric distress and would have greater rates of formal anxiety and depression diagnoses. Furthermore, we predicted that higher CGG repeat lengths and elevated mRNA levels would be associated with higher levels of psychiatric distress, especially anxiety and depression.

MATERIALS AND METHODS
Participants

The study protocol was approved by the Institutional Review Board at the University of California, Davis Medical Center. Approximately 900 pedigrees of families affected by fragile X were screened to identify women previously known to carry the FMR1 premutation (55–200 CGG repeats) or women who were subsequently offered testing based on their known risk to be a carrier. Additional

participants were recruited through flyers posted by the National Fragile X Foundation. Only women without children or with children confirmed negative for FXS or any disability were enrolled. None of the women with the premutation were clinic referred or selected for recruitment based on any criteria other than the premutation status. Many of the participants were sisters, daughters, or cousins of mothers with children with FXS. All participants were between the ages of 18 and 50 years, and were neurologically unaffected. For a comparison group, females within the same age range were drawn from the general population through the MIND Institute participant registry and from use of flyers posted at the University, and were subsequently confirmed to have normal *FMR1* alleles (<45 CGG repeats). Individuals with drug/alcohol abuse or dependence were excluded. At screening and consent for the overall study, participants were informed that "we hoped to learn more about the structure and activity of the brain related to memory and emotion in individuals with the fragile X premutation in comparison to those who do not carry the premutation."

On all demographic data, the premutation group and the control group were similar, with no significant differences. The average age for the premutation group was 32.14 years (SD = 7.15), and for the control group 30.52 years (SD = 8.03). The overall sample comprised 6% Asian, 2% African-American, 12% Hispanic, 72% Caucasian, 6% more than one race, and 2% unknown or not reported. There were no group differences in marital status, household income, education level, ethnicity, or age. These descriptive statistics are illustrated in Table I.

At the time of assessment, 8 of the 26 premutation carriers (30.8%) and 0 (0%) controls were being treated with selective serotonin/serotonin-norepinephrine reuptake inhibitors (SSRI/SNRI). Other psychoactive medication use included: three carriers (11.5%) and zero (0%) controls were treated with benzodiazepines; one carrier (3.8%) and zero (0%) controls were taking an anticonvulsant medications; one carrier (3.8%) and zero (0%) controls were prescribed sleep aids; and one control (4%) and zero (0%) of carriers were treated with psychostimulants.

Molecular Genetic Measures

CGG repeat size. Genomic DNA was isolated from peripheral blood lymphocytes using standard methods (Qiagen, Valencia, CA). Repeat size and methylation status were determined using both polymerase chain reaction and Southern blot analysis using an Alpha Innotech FluorChem 880 Image Detection System (San Leandro, CA) as previously described [Tassone et al., 2008; Filipovic-Sadic et al., 2010].

FMR1 mRNA. *FMR1* mRNA expression levels were determined by qRT-PCR as previously described [Tassone et al., 2000a]. Levels of *FMR1* mRNA were missing for one participant (premutation carrier).

Psychological and Psychiatric Measures

Structured clinical interview for DSM-IV axis I disorders. The SCID-I is a clinician administered, semi-structured interview, covering a broad range of psychiatric diagnoses according to DSM-IV criteria [First et al., 1997]. The SCID was customized for this study to include the mood, anxiety, somatoform, and adjustment disorders modules, as well as the psychotic symptom screener. Lifetime and current psychiatric diagnoses were noted. All SCID interviews were completed by a psychiatrist or a psychologist with SCID training to ensure administrations were standardized across examiners. The interviewers were kept blind to participant group in the majority of cases, except when participants' comments during interviews made group membership to be known occasionally.

Symptom checklist-90-revised. The SCL-90-R is a standardized self-report measure of psychiatric symptoms occurring over the past week [Derogatis, 1994]. Ninety questions are clustered into the following symptom dimensions: somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. A Global Severity Index (GSI) is an indicator of overall level of psychiatric disturbance. Internal reliability coefficient alphas range from a low of 0.77 to a high of 0.90 across scales and 1-week test–retest reliability ranges from 0.80 to 0.90. Details of the internal structure and convergent-discriminant validity of the instrument are reported in the SCL-90-R manual [Derogatis, 1994].

Beck anxiety inventory. The Beck Anxiety Inventory is a 21-item self-report questionnaire including typical symptoms of anxiety during the past week such as nervousness, inability to relax, and heart pounding or racing [Beck et al., 1988]. It has high internal consistency (Cronbach's alphas ranging from 0.90 to 0.94) and test–retest reliability over a 1-week interval (0.67–0.93). It has demonstrated good convergence with other measures of anxiety in adults in psychiatric and community samples.

Data Analysis

Group differences. To examine differences in self-reported psychiatric symptoms, we carried out a MANOVA with *FMRI* status (premutation vs. control) as the independent variable and subscales of the SCL-90-R as dependent variables. Follow-up *t*-tests were completed to determine which subscale means differed by group. A Benjamini–Hochberg adjustment for false discovery rate was applied to *P*-values for the SCL-90-R and Beck Anxiety scores combined (all self-report rating scales). In order to cover a broader range of anxiety symptoms, both the BAI and the SCL-90-R Anxiety Subscale were used for analysis. For the SCID group differences, we coded each disorder as present or absent according to the DSM-IV criteria for each participant, and carried out chi-squared tests. The lifetime rates of each disorder are reported, in contrast to the levels of current psychiatric problems captured by the SCL-90-R and Beck Anxiety Inventory (Table II). We completed Pearson's correlations to examine the associations between *FMR1* molecular measures (corrected for AR) and the dimensional measures of psychiatric symptoms (SCL-90-R and BAI). Analyses were carried out using SPSS and R software [IBM Corp, 2015; R Core Team, 2016].

The correlation analysis between CGG repeat size and psychiatric symptom severity took into account the protective effects of the normal X chromosome expressed by the AR, which indicates the proportion of cells carrying the normal allele on the active X chromosome. Thus, the data were corrected for the influence of the AR as previously described [Tassone et al., 2000b].

TABLE I. Demographic and *FMR1* Molecular Characteristics by Group

	Control (n = 24)	Premutation (n = 26)	C versus P t, χ^2 (df, *P*)
CGG[a] repeat, M (SD), range	31.2 (2.7), 28–39[b]	89.5 (21.7), 60–152	
FMR1 mRNA, M (SD), range	1.3 (0.3), 0.5–1.7	2.3 (0.5), 1.5–3.6[c]	
Age (years), M (SD)	30.52 (8.03)	32.14 (7.15)	0.76 (48, 0.45)
Race/ethnicity (%)			7.04 (5, 0.22)
White/Caucasian	58.3	84.6	
Hispanic	20.8	3.8	
Asian	3.8	3.8	
Black/African-American	0.0	3.8	
More than one race	8.3	3.8	
Unknown/not reported	4.2	0.0	
Marital status (%)			1.33 (2, 0.51)
Never married	45.8	53.8	
Married	41.7	42.3	
Divorced	12.5	3.8	
Education (%)			7.19 (5, 0.21)
High school diploma/GED	0 .0	3.8	
Some college	29.2	3.8	
Associates degree	4.2	3.8	
Bachelor's degree	37.5	46.2	
Some graduate/professional	12.5	11.5	
Graduate/professional	16.7	30.8	
FSIQ, M (SD)	116.4 (11.8)	119.1 (13.9)	−0.73 (47, 0.47)
Years attended school, M (SD)	16.7 (2.3)	17.7 (3.6)	1.13 (46, 0.26)
Household income (%)			2.48 (6, 0.87)
<$25,000	16.7	15.4	
$25–50k	33.3	30.8	
$50–75k	20.8	15.4	
$75–100k	0.0	3.8	
$100–150k	8.3	3.8	
$150–250k	16.7	19.2	
Prefer not to say	4.2	11.5	

M, mean; SD, standard deviation.
[a]CGG repeat size was calculated using the larger of the two X chromosome alleles.
[b]CGG repeat size was calculated for 23 control participants.
[c]*FMR1* mRNA was calculated for 25 subjects with the premutation.

TABLE II. Means, Standard Deviations, and Results of Group Comparisons for SCL-90-R and Beck Anxiety Scale

	Control (n = 24)	Premutation (n = 26)	
Scale	M (SD)	M (SD)	t (df, *P*)
Interpersonal sensitivity	50.46 (10.42)	60.23 (9.49)	3.47 (48, 0.001*)
Depression	49.00 (11.18)	57.38 (8.12)	3.05 (48, 0.004*)
Hostility	47.13 (7.47)	53.42 (9.55)	2.58 (48, 0.013)
Somatization	46.58 (8.37)	54.19 (8.93)	3.04 (48, 0.004*)
Anxiety	45.96 (9.15)	54.85 (11.51)	3.01 (48, 0.004*)
Phobic anxiety	46.92 (8.32)	51.00 (9.15)	1.65 (48, 0.106)
Paranoid ideation	46.96 (8.60)	48.31 (8.12)	0.57 (48, 0.571)
Obsessive-compulsive	51.71 (9.28)	60.50 (8.03)	3.59 (48, 0.001*)
Global severity index	47.63 (11.31)	57.62 (9.12)	3.45 (48, 0.001*)
Beck anxiety inventory	49.92 (7.11)	60.96 (13.07)	3.75 (39.23, 0.001*)

M, mean; SD, standard deviation.
*Significant after Benjamin–Hochberg false discovery rate adjustment.

TABLE III. Means, Standard Deviations, and Results of Chi-Squared tests for SCID Depression and Anxiety Diagnoses Comparing Women With the Premutation to Controls

SCID diagnosis	Control (n = 24) n (%)	Premutation (n = 26) n (%)	Control versus premutation χ^2 (df, P)
Dysthymic disorder	3 (12.5)	4 (15.4)	NA
Major depressive disorder	11 (45.8)	15 (57.7)	0.70 (1, 0.50)
Depressive disorder NOS	1 (4.2)	1 (3.8)	NA
Any depressive disorder	13 (54.2)	15 (60.0)	0.17 (1, 0.68)
Panic disorder without agoraphobia	2 (8.3)	6 (23.1)	NA
Agoraphobia without panic	1 (4.2)	0 (0)	NA
Social phobia	3 (12.5)	11 (42.3)	5.50 (1, 0.12)
Specific phobia	10 (41.7)	8 (30.8)	0.64 (1, 0.50)
Obsessive-compulsive disorder	1 (4.2)	4 (15.4)	NA
Posttraumatic stress disorder	2 (8.3)	3 (11.5)	NA
Generalized anxiety disorder	3 (12.5)	8 (30.8)	2.43 (1, 0.36)
Any anxiety disorder	15 (62.5)	19 (76.0)	1.05 (1, 0.50)

NA, not applicable due to insufficient counts to allow for comparisons; NOS, not otherwise specified; SE, standard error.

RESULTS

Symptom Check List-90-Revised (SCL-90-R) and Beck Anxiety Inventory

The internal consistency of the SCL-90-R in this sample was very strong (Global Index, $\alpha = 0.98$; subscale α's ranged from 0.73 for somatization to 0.91 for depression). Assumptions were met for independence, normality, and homogeneity of variances. Results from the MANOVA showed a significant overall group difference, $F (9, 40) = 3.49$, $P = 0.003$. The means and standard deviations used for this analysis and the results of the follow-up t-tests are presented in Table II. Females with the premutation reported experiencing significantly elevated symptoms relative to controls on all scales with the exception of hostility, phobic anxiety, and paranoid ideation, after adjusting for multiple comparisons. The internal consistency of the BAI was also good ($\alpha = 0.87$). Women with the premutation also reported significantly elevated anxiety than controls on the BAI, $t (48) = 3.75$, $P = 0.001$. The mean score of the premutation group was over one standard deviation above the mean scores for the control group on the BAI ($M_P = 60.96$, $M_C = 49.92$).

Correlations Between FMR1 Measures and Self-Reported Psychiatric Symptoms

Within the premutation carrier group, all correlations between molecular (AR-corrected CGG and FMR1 mRNA) and SCL-90-R and BAI scores were not significant (all $P > 0.14$; all $r < 0.34$).

Structural Clinical Interview for DSM Diagnoses (SCID)

Chi-squared tests of independence were used to determine whether there was a difference in rate of each diagnosis as a function of group. The number of participants within each group for several of the individual anxiety and depression diagnoses were not adequate to complete the chi-squared analyses (NA shown in Table III). However, we were able to perform post-hoc analyses after combining all anxiety disorder (panic disorder without agoraphobia, agoraphobia without history of panic disorder, social phobia, specific phobia, obsessive-compulsive disorder, posttraumatic stress disorder, and generalized anxiety disorder) and all depressive disorder (dysthymic disorder, major depressive episode, depressive disorder NOS) diagnoses into the categories: any anxiety and any depression diagnoses. These results are shown in Table III.

For individual disorders with adequate sample sizes, chi-squared tests showed a significantly higher rate of social phobia in premutation carriers (42.3%) relative to controls (12.5%), $\chi^2 (1, N = 50) = 5.50$, $P = 0.02$. However, after correction for multiple comparisons, this difference was no longer significant ($P = 0.12$). The frequencies of any depression diagnosis, $\chi^2 (1, N = 49) = 0.17$, $P = 0.68$ and any anxiety diagnosis, $\chi^2 (1, N = 49) = 1.05$, $P = 0.50$ did not differ significantly by group, although the rates of these in the control group were very high, at 54.2% and 62.5%, respectively.

DISCUSSION

The present study provides preliminary evidence that significant depression, somatization symptoms, interpersonal sensitivity, obsessive-compulsive symptoms, and anxiety occur at high rates in this unselected, non-referred sample of women with the FMR1 premutation who are not mothers of children with FXS. Eleven of the 26 (42.3%) women with the premutation had a lifetime history of social phobia, which was significantly higher than our control group. Although this difference was not significant after adjustment for multiple comparisons, the premutation rate was over three times the rate in women in the general population and our control group reported a higher than normal rate of this disorder. The high rate of social phobia is consistent with prior studies of

premutation carriers of both genders [Bourgeois et al., 2011] and with the earlier study of females only by Franke et al. [1998]. An especially insightful study by Hunter et al. [2012] showed that genetic variance within corticotropin-releasing hormone receptor gene (CRHR1) moderates the parenting stress of raising a child affected by FXS to impact the severity of social phobia symptoms among women with the fragile X premutation. Although formal diagnostic psychiatric interviews were not used, a clinical cutoff on the social phobia scale used for the study resulted in similar findings. Thus, it is of interest that we observed high rates of diagnosed social phobia among women with the premutation without affected children. Combined, the two studies suggest a special vulnerability to social anxiety that is exacerbated by both secondary genes impacting the stress response as well as ongoing stressors related to raising affected children. However, it is important to note that Roberts et al. [2009], using the same diagnostic interview as reported here (SCID), but in a group of premutation carriers with affected children, reported a significantly lower lifetime rate of social phobia (7.53%) but an elevated rate of major depression (43.01%), compared to the general population. Thus, the emergence and symptomatic expression of mood and anxiety disorders in women with the premutation appears to be shaped by both secondary genetic and family environmental factors.

A recent study by Roberts et al. [2016] found changes in the psychological profile of mothers with the premutation over time, specifically noting that lifetime major depression disorder (MDD) increased from 46% to 54%, and anxiety disorders increased from 28% to 35% across a 3-year span. Women with midrange CGG repeat size were found to be at a moderately higher risk for developing MDD, indicating an inverse relationship between CGG repeat length and psychiatric symptom severity [Roberts et al., 2016]. Although this study found FXPOI to be highly prevalent (41%), this factor failed to account for the elevated rates of psychiatric disorders, as rates of MDD and anxiety disorders did not differ between women over age 40 with and without FXPOI. Overall this study identified specific risk factors for the development of psychological disorders, including midrange CGG repeat length, elevated child problem behaviors, and unmarried status.

Although the rates of other formal Axis I disorders did not differ from the control group, we noted that the premutation group rates of these disorders were considerably higher than general population estimates of women using the National Comorbidity Survey Replication [NCS-R; Kessler et al., 2005]. Indeed, the rates of several anxiety and depressive disorders are two to three times higher than the NCS-R population prevalence estimates. We also emphasize that eight of the female premutation carriers (30.8% of the sample) were taking some kind of psychoactive medication, compared to zero controls. With such a high proportion of premutation carriers on psychoactive medication, it is plausible that current psychiatric symptoms and/or disorders would have been even greater in this group, had this subgroup not been on active medication at the time of the study. Our control group did not adequately represent the general population in terms of psychiatric illness, which likely explains the lack of significant differences between groups for several disorders. This may reflect regional and/or cohort differences between our controls and the national sample, with some controls perhaps seeking care by participating in research. For example, the rate of lifetime major depressive disorder in our control group was 46% compared to 20% in the general population. However, the SCL-90-R and BAI detected significant elevations in a range of symptoms of premutation carriers over the past week, relative to controls. Thus, there may be more common subclinical psychiatric symptoms in many carriers that fall below SCID criteria for a formal diagnosis.

Our prior work examining psychiatric symptoms (using the SCL-90-R) and *FMR1* molecular measures in an older and larger cohort of women with the premutation did not demonstrate an association with CGG repeat size, mRNA, or FMRP, as measured by immunocytochemistry staining of lymphocytes [Hessl et al., 2005]. In that study, we did find a significant correlation between elevated mRNA and anxiety in the subgroup of females with skewed AR toward active premutation alleles. In the current study cohort, though much smaller, we found no such effect by examining AR-adjusted CGG size. Although FMRP measurements are not available in the current study, the prominence of social phobia suggests that subtler FMRP deficits may be contributory to these symptoms in some women with the premutation—indeed, social anxiety is a phenotypic feature of the full mutation, which is caused by much more substantial FMRP deficits. The role of FMRP levels in premutation carrier expression of psychiatric disorder may be an important focus of future research. For example, our preliminary fMRI studies in male premutation carriers suggest that FMRP is associated with limbic system and social-emotional functioning [Hessl et al., 2011]. However, given the lack of robust genotype–phenotype correlations for psychopathology in women with the premutation, we suggest that other secondary genetic [e.g., Hunter et al., 2012] and/or environmental factors may be involved, requiring more complex statistical modeling and larger samples than was possible here. For example, two studies of independent cohorts demonstrated a non-linear association between molecular measures (CGG repeat length) and psychiatric symptoms, especially depression and anxiety [Seltzer et al., 2012; Loesch et al., 2015], and several studies have detected a similar non-linear effect of CGG length on severity of FXPOI symptoms [Sullivan et al., 2005; Allen et al., 2008; Hunsaker et al., 2011]. Furthermore, in this study sample, visual inspection of scatter plots indicated the possibility of a non-linear relationship between psychiatric distress and *FMR1* mRNA; however, sample size limited our ability to more formally test these associations, and thus we caution against any firm conclusions about the lack of such associations observed in this relatively small study.

The study was limited by small sample sizes, likely limiting generalizability to the larger population of women with the premutation. However, the uniqueness and importance of the cohort, excluding mothers of children affected by FXS, elevate the potential novelty and importance of the observations. Although the female carriers in the study were recruited without regard to clinical concerns, there is likely to be recruitment bias, as those who choose to travel to our site for research may have been more likely to be seeking medical or psychiatric care or to be motivated by helping their affected family members. However, females with more severe psychiatric symptoms, particularly social phobia, may be less likely or able to engage in the research. Similarly, the exclusion of participants with a history of alcohol or drug abuse may also

screen out individuals with more severe or complex psychiatric involvement. Finally, we do not have information regarding whether any of the women in the study had FXPOI or were undergoing hormone fertility treatments. It is possible that fertility issues play into psychiatric symptoms of depression, anxiety, and stress in this population. This is an important area for future research.

Our data, when considered in light of prior reports of anxiety and depression in carriers, suggest that the premutation appears to confer additional risk for mood and anxiety disorders, which, when combined with parenting and other stressors associated with raising children with FXS, may lead to yet greater susceptibility of these disorders in young mothers. Thus, a careful screening by the medical practitioner for histories of psychiatric disorder in all women with the premutation is warranted. This screening may be especially critical for women with the premutation planning to have children, and certainly after having a child with FXS, as any predisposing depression or anxiety symptoms can be treated earlier. This early treatment is important given the known association between parent psychopathology and maladaptive behaviors in children with FXS [Hessl et al., 2001]. In addition to screening for psychiatric disorders in young mothers, it is also important to continue to screen for the presence of mood and anxiety disorders in premutation carriers throughout the lifetime, as the age of onset of symptoms in premutation carriers has been found to be significantly later for major depression, panic disorder, and specific phobias, than in the general population [Seritan et al., 2013].

To date, there are no premutation-specific empirically validated treatments for psychiatric disorders. Currently, treatment for mood and anxiety disorders in carriers should be addressed as it is with the adult general population with cognitive behavioral therapy, pharmacological treatment if needed, and exercise, for example. The use of medication for treatment of anxiety, depression, and obsessive compulsive symptoms for women with the premutation appears to be effective [Polussa et al., 2014]. In addition, SSRI can stimulate neurogenesis in an aging brain, thus may be neuroprotective against later cognitive decline [Jacobs et al., 2000; Santarelli et al., 2003; Hagerman et al., 2009; Polussa et al., 2014], which may be relevant for protection against FXTAS and other neurological symptoms related to the premutation. Confirmation of this speculation awaits further study.

Future research using larger sample sizes will help shed further light upon the results presented in this study. Samples that include women with the premutation with and without affected children, control mothers of children with other forms intellectual disability, and mothers of children without disabilities as additional comparison groups may help further tease apart the impact of raising an affected child from more direct impacts of the *FMR1* premutation on emotional and physical health.

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