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Investigation of Phenotypes Associated with Mood and Anxiety Among Male and Female Fragile X Premutation Carriers

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Abstract

The fragile X disorder spectrum, due to a CGG expansion in *FMR1*, includes fragile X syndrome (>200 repeats) and the premutation-associated disorders of ovarian insufficiency and tremor/ataxia syndrome (~55–199 repeats). Altered neurobehavioral profiles including variation of phenotypes associated with mood and anxiety may be expected among younger premutation carriers given this spectrum of disorders. However, previous studies have produced conflicting findings, providing

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the motivation to examine these phenotypes further. We investigated measures of mood and anxiety in 119 males and 446 females age 18–50 ascertained from families with a history of fragile X syndrome and from the general population. Scores were analyzed using a linear model with repeat length as the main predictor, adjusting for potential confounders. Repeat length was not associated with anxiety, but was marginally associated with depression and negative affect in males and negative affect only in females. These results suggest that premutation carriers may be at risk for emotional morbidity; however, phenotypic differences were subtle and of small effect size.

Keywords

FXTAS; FMR1; Premutation; CGG repeat; Neurobehavior; Depression; Anxiety

Introduction

Fragile X mental retardation 1 gene (*FMR1*) is located near the end of the long arm of the X chromosome and contains a highly polymorphic CGG repeat in the 5′ UTR of exon 1. The most common alleles for *FMR1* contain fewer than 40 repeats (Snow et al. 1993). In rare cases, the repeat can become unstable and expand. If the repeat number exceeds 200, termed full mutation, the gene becomes hypermethylated and no gene product, FMRP, is made due to transcriptional silencing (Sutcliffe et al. 1992). This loss of FMRP is responsible for fragile X syndrome (FXS), the most common identified form of heritable mental retardation (Pieretti et al. 1991). FXS has a prevalence of roughly 1 in 4,000 for males and 1 in 8,000 for females (Crawford et al. 2001). The clinical presentation of males with FXS is variable, but the most common phenotypes include mild to severe mental retardation, developmental delay, hyperactivity, social anxiety and other anxiety disorders, and autistic-like features. As a group, females are more mildly affected due to X-inactivation (Reiss and Dant 2003).

Intermediate alleles, about 45–54 repeats, may or may not be stable during transmission from parent to child and do not expand to a full mutation in one generation. Premutation alleles are defined as unmethylated alleles with repeat numbers in the range of 55–199 that are unstable during transmission and can lead to a full mutation in one to three generations (Maddalena et al. 2001). The smallest repeat to expand to a full mutation in one generation is 59 repeats (Nolin et al. 2003). About 1 in 250 females and 1 in 800 males carry one of these high repeat alleles (Crawford et al. 2001). Premutation alleles remain unmethylated, therefore *FMR1* is transcriptionally active and produces FMRP. *FMR1* mRNA levels linearly increase across the premutation range due higher rates of transcription as a results of a mechanism that is presently not understood (Tassone et al. 2000a, b; Kenneson et al. 2001; Tassone and Hagerman 2003; Allen et al. 2004; Garcia-Alegria et al. 2007). However, a negative association has been found between FMRP and repeat size in premutation carriers due to a decreased translation efficiency of the mRNA as the repeat size increases (Feng et al. 1995; Tassone et al. 2000b; Kenneson et al. 2001; Primerano et al. 2002; Tassone and Hagerman 2003).

Two phenotypes are associated with these premutation alleles. Males with the premutation who are over the age of 50 are at risk for a neurodegenerative tremor/ataxia syndrome (FXTAS). This disorder is very rare in females who carry the premutation allele. However, female carriers of the premutation are at an increased risk of primary ovarian insufficiency (FXPOI) (Sherman 2000; Abrams 2007; Welt 2007). FXTAS and FXPOI have not been found to be associated with the full mutation, thus they are not associated with a lack of the *FMR1* protein product. For FXTAS, converging evidence indicates that the phenotype is a

result of the toxic effect of the expanded repeat length in the *FMR1* mRNA (Hagerman and Hagerman 2004).

Numerous studies have investigated neuropsychological phenotypes among premutation allele carriers. Conflicting results have been reported and a definitive profile fails to emerge (for review, see Hunter et al. submission). Most of these studies were conducted prior to the identification of FXTAS, have primarily utilized small samples with varying ascertainment methods and phenotype measurement modalities, lack proper controls, and concentrate solely on female premutation carriers. The use of female study populations makes interpretation of the results difficult due to the X-linked nature of *FMR1.*

Several studies have concluded that the premutation among females lacks a detectable neuropsychological phenotype (e.g., (Reiss et al. 1993; Thompson et al. 1994; Bennetto et al. 2001)). Other studies, some with both males and females participants, have concluded that premutation allele carriers manifest milder forms of clinical features seen in FXS, including learning disabilities, cognitive deficits, developmental delay, and attention deficits, as well as physical features such as prominent ears and flexible finger joints (e.g., (Hull and Hagerman 1993; Cornish et al. 2005)). One study suggested that premutation allele carriers may to be at a higher risk of autism spectrum disorders (Aziz et al. 2003), although this has not been confirmed.

An increased risk of anxiety and mood disorders among premutation allele carriers has not been established. Some studies have reported a lack of phenotype (e.g., (Reiss et al. 1993; Sobesky et al. 1996)), while others have reported repeat length associations with psychiatric symptoms (e.g., (Franke et al. 1998; Johnston et al. 2001)). More recently, Hessl et al. (2005) found that *FMR1* transcript level, but not repeat length or FMRP levels, was significantly associated with increased severity of psychiatric symptoms in males, independent of FXTAS status.

In 2005 we published a study examining cognition among 66 men and 217 women with varying *FMR1* repeat lengths (Allen et al. 2005). We reported that women who were carriers of premutation alleles had significantly lower verbal IQ scores compared to non-carriers. Here, we examine phenotypes associated with mood and anxiety among carriers of fragile X premutation alleles in the largest study population to date consisting of 119 men and 446 women. All study participants were between the ages of 18 and 50 at the time of testing. Thus, any phenotypes detected here would most likely not be due to the presence of FXTAS, but would potentially indicate a more global impairment among premutation carriers in general.

Methods

Study population

A large sample of study participants were recruited from the general population and from families with a history of FXS. The study population was the result of a 78% participation rate and included males and females with repeat sizes ranging from 20 to 180. Participants from the general population were recruited from Atlanta area hospitals, churches, universities, technical schools, corporations, sports events, and health fairs. Recruitment from families with a known history of FXS was pursued to enrich the sample population with carriers of expanded alleles. FXS families were identified through clinics, internet postings, FXS parent groups, and word of mouth. Participation was limited to those aged 18–50 years whose primary language was English. The majority of participants were unrelated, while some were ascertained from the same pedigree. In the female sample, there were 47, 14, 8, 3, and 2 families with 2, 3, 4, 6, and 7 female participants, respectively. In

the male sample, there are 11, 1, 1, and 1 families with 2, 3, 4, and 5 participants, respectively. The remaining were singletons. Thus, overall 446 women were ascertained from 320 families and 119 men from 99 families. The protocols and consent forms for ascertainment were approved by the Institutional Review Board at Emory University. For more information on study population ascertainment, see Allen et al. (2005).

In an effort to create roughly equal sized groups for analysis, particularly for males, we used the following allele group definitions: intermediate allele = 41–60 repeats and premutation allele = 61–199 repeats. Although these differ slightly from those proposed for a clinical application (i.e., those based on risk for instability) (Sherman et al. 2005), they are similar to previous studies used to examine *FMR1* mRNA levels (Allen et al. 2004; Garcia-Alegria et al. 2007). At this point in time, there is no biological underpinning for any of these definitions, particularly with respect to risk for neuropsychological or neurobehavioral phenotypes. Thus, we used those outlined above to better balance sample sizes.

Data collection

Each study participant was asked to complete a medical history questionnaire and a neuropsychological test battery that included the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) to determine IQ scores as well as several widely-used self-report inventories of mood and anxiety described below. Test administrators were blind to the subject's *FMR1* genotype as well as family history of FXS. For molecular analysis to determine CGG repeat size of *FMR1*, participants were asked to provide a blood or buccal brush sample.

Measurement of IQ and phenotypes associated with mood and anxiety

Symptoms of depression were measured with The Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977). The CES-D consists of 20 items rated on a four-point scale, indicating how frequently each symptom was experienced in the past week (0 = rarely or none of the time, 1 = some or a little of the time, 2 = occasionally or a moderate amount of time, and 3 = all of the time). Total scores can range from 0 to 60, with higher scores indicating higher levels of emotional distress associated with depression. Scores of 16 or more suggest clinically significant depression. The CES-D has high internal consistency, with a value of about 0.85 for the general population and about 0.91 for a patient sample. The test–retest reliability is moderate with a value of about 0.58. CES-D scores were obtained for all participants.

The State-Trait Anxiety Inventory (STAI) is a two-part inventory used to measure levels of current anxiety (state anxiety) and general anxiety susceptibility (trait anxiety) (Spielberger 1983). Each subscale consists of 20 items, each rated on a four-point scale. The state anxiety subscale measures the severity of current anxiety symptoms (1 = not at all, 2 = somewhat, 3 = moderately so, and 4 = very much so). The trait anxiety subscale measures the frequency of anxiety symptoms experienced in general (1 = almost never, 2 = sometimes, 3 = often, and 4 = almost always). STAI state and trait anxiety scores range from 20 to 80, with higher scores indicating higher levels of anxiety. The STAI has good internal consistency, ranging from 0.86 to 0.96. Test–retest reliability is highly dependent on the subject population and can range from 0.65 to 0.86 for the trait anxiety subscale and 0.16–0.62 for the state anxiety subscale. This inventory was added to the test battery after the initiation of study participant recruitment, thus state anxiety and trait anxiety scores for 54 male and 174 female participants were not obtained.

The Social Phobia and Anxiety Inventory (SPAI) is a two-part inventory used to measure symptoms of social phobia in various social situations (Turner and Beidel 1996). The social

phobia subscale consists of 32 items rated on a seven-point scale indicating how frequently symptoms of social phobia are experienced in various social situations (0 = never to 6 = always). The agoraphobia subscale consists of 13 items rated on the same seven-point scale. By subtracting the social phobia and agoraphobia subscores, this test is capable of distinguishing pure social phobia from social distress due to panic disorder with agoraphobia. Higher subscale scores and "difference" scores reflect higher levels of anxiety. An agoraphobia subscale score of 39 or above is indicative of possible panic disorder, while a "difference" score of 80 or above is indicative of probable social phobia. The SPAI has high internal consistencies with a value of 0.96 for the social phobia subscale and 0.85 for the agoraphobia subscale. Test–retest reliability ranges from 0.74 to 0.86, depending on the subscale. SPAI scores for two female participants were incomplete and thus unavailable for analysis.

General and specific emotional states were measured with The Positive and Negative Affect Schedule (PANAS), a 60-item scale (Watson and Clark 1994). Two broad affective states, negative and positive, are each measured by 10 items, all on a five-point scale indicating the extent to which each emotion was felt in the past year (1 = very slightly or not at all, 2 = a little, 3 = moderately, 4 = quite a bit, and 5 = extremely). Using the same five-point scale, the remaining 40 items are used to measure 11 specific affective states: fear, sadness, guilt, hostility, shyness, fatigue, surprise, joviality, self-assurance, attentiveness, and serenity. The PANAS has internal consistencies ranging from 0.72 to 0.94, depending on the subscale and study population. Test–retest reliabilities range from 0.51 to 0.68. The PANAS questionnaire was incomplete for one male subject, and thus his subscale scores were not available.

Lastly, each subject's full-scale IQ was measured as part of the neuropsychological test battery using the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) (Wechsler 1997).

Laboratory methods

FMR1 CGG repeat number—Each study participant was asked to provide a blood or buccal brush sample for molecular analysis. For more information on molecular analysis, see Allen et al. (2005). Briefly, DNA was extracted from samples with the Qiagen QiAmp DNA Blood Mini Kit. A fluorescent-sequencer method using an ABI Prism 377 DNA sequencer was used to determine *FMR1* CGG repeat length (Meadows et al. 1996). When no repeat length band for males or only one band for females was present, an alternative PCR-based, hybridization technique was used to identify larger premutation or full mutation alleles (Brown et al. 1993). For heterozygous females, CGG repeat length from the larger repeat allele was used in subsequent statistical analyses.

Statistical analysis

Descriptive statistics for the study population are shown in Table 1, with male and female data shown separately. The demographic variables included age at the time of testing (continuous variable), ethnicity (dichotomous variable: 0 = Caucasians and Asians, 1 = other ethnicities), education level reached at the time of testing (dichotomous variable: 0 = high school completed or less, 1 = some college completed or more), household income level at time of testing (dichotomous variable: 0 = less than $50,000, 1 = $50,000 or more), full-scale IQ (continuous variable), method of ascertainment (dichotomous variable: 0 = recruited from families with a known history of FXS, 1 = recruited from the general population), and anxiety or depression medication use at the time of testing (dichotomous variable: 0 = not taking anxiety/depression medications, 1 = taking anxiety/depression medications). Analysis of variance was used to test for repeat length group differences for

continuous demographic variables while chi square tests were used for dichotomous demographic variables. Significant differences between repeat length groups were noted for race and ascertainment source among male participants and for these same two variables plus age at testing, level of household income, and the use of anxiety and/or depression medication at the time of testing for female participants (Table 1). Thus, all models for emotional outcomes were adjusted for age, income, medication use, race, and ascertainment source.

For each test analyzed, males and females were modeled separately due to the X-linked nature of *FMR1*. The distributions of scores for each measure were tested for normality. Scores were transformed, if necessary, to produce a normal distribution for further analysis. A natural logarithm transformation was needed for the STAI state and trait anxiety and for the PANAS general negative affect scores. A square root transformation was required for the CES-D and the SPAI social phobia and agoraphobia scores.

Scores were analyzed using general linear regression equations modeled for correlated outcomes. This approach was used to adjust for correlated data that may have occurred among relatives from the same family due to shared environmental or genetic factors. In addition, this approach is robust to the varying family cluster sizes among our sample population. Length of the *FMR1* repeat was used as the main predictor of mood and anxiety scores and was classified in two ways. First, repeat length was used as a continuous variable. Second, subjects were divided into three groups based on their repeat length: non-carriers (40 repeats or less), intermediate allele carriers (41–60 repeats) and premutation allele carriers (61–199 repeats). In this analysis, repeat length classes were used as the predictor with the non-carrier group as the reference group. A Tukey's post hoc analysis was performed to identify differences in adjusted mean scores among repeat length groups. All interaction terms that consisted of a covariate and repeat length, either as a continuous or as a class variable, were tested for each model.

The psychosocial stress of raising a child with FXS could contribute to any emotional morbidity detected in our analyses. This possibility was addressed in two ways. First, the analyses were repeated including adjustment for having a FXS child. Second, premutation carriers were divided into two groups: those with a FXS child and those without a FXS child. Analysis of covariance (ANCOVA) was used to test for mean score differences between the two groups.

Although many statistical tests were performed, adjustment for multiple testing was not straightforward due to the correlation among the eight mood and anxiety outcome scores. Further, scores were tested in two consecutive models, one with repeat length as a continuous variable and one with repeat length as a categorical variable, so these tests cannot be considered independent due to the correlation between these two repeat length variables. Thus, we present the results using a significance level of $P<0.05$, but provide all P-values, and discuss the results in this context. Further discussion of the influence of multiple testing on interpretation of results is provided in Section “Discussion”. In addition, we calculated the effect size for each significant mean score difference between repeat length groups using Cohen's d score (Cohen 1992). According to Cohen, values of 0.2, 0.5, and 0.8 indicate small, medium, and large effect sizes, respectively (Cohen 1992). All statistical analyses were performed using the PROC MIXED procedure on the SAS System for Windows, Release 8.2.

Results

For males, positive associations were detected between repeat length and depression scores (CES-D, $P = 0.03$; Table 2) and general negative affect scores of the PANAS ($P = 0.04$, Table 2). No associations were observed for repeat length and state anxiety, trait anxiety, positive affect, social phobia, or agoraphobia scores (Table 2). Using repeat length group as a predictor, no mean score differences among repeat length groups were seen for any outcome measure (Table 3). For all models, all interaction terms for the repeat length were tested to analyze any modifier effects of the confounders. No interaction terms were significant for any model for any confounder, including age.

For females, a positive association was seen between repeat length and general negative affect scores ($P = 0.04$; Table 2), similar to the results among males. This association was also indicated when repeat length group was used as a predictor variable: premutation carriers scored higher than non-carriers although the effect size was small (Cohen's $d = 0.36$, $P = 0.02$; Table 3). However, unlike males, there was no association observed with depression scores. No associations were observed for state anxiety, trait anxiety, positive affect, social phobia, or agoraphobia scores (Table 2) nor were there any group mean differences (Table 3).

The PANAS also provides subscores for specific emotions. In order to follow up on the association of repeat length and general negative affect among males and females, the subscores were analyzed for all of the specific negative emotions tested by the PANAS: fear, sadness, guilt, and hostility. Results of this analysis are shown in Tables 4 and 5. Among males, positive linear associations were detected with sadness ($P = 0.03$) and guilt ($P = 0.01$), but not fear or hostility. In addition, the premutation group had a higher mean score for guilt compared to the non-carrier group, with a medium effect size (Cohen's $d = 0.78$, $P = 0.03$). Among females, linear associations with repeat size were not detected for any of the four specific emotion scores. However, the premutation group did have higher mean scores for fear (Cohen's $d = 0.30$, $P = 0.05$) and hostility (Cohen's $d = 0.28$, $P = 0.05$) compared to the non-carrier group, though with small effect sizes.

To investigate the clinical implications of scores, diagnostic rates provided by the relevant measures were analyzed. The CES-D measure provides a cutoff value for the diagnosis of probable depression, while the SPAI provides cutoff values for probable social phobia and probable panic disorder. Although the means did not exceed the diagnostic cutoff for any of the repeat length groups, the distribution of the frequency of participants who scored above this cutoff score was examined (Table 6). For probable depression, the rates differed by group for males (Fisher's Exact test: $P = 0.0093$), but not females. For probable social phobia, the frequency of those exceeding the cutoff increased with increasing repeat length group for females (Fisher's Exact test: $P = 0.0004$). Finally, for probable panic disorder, premutation males had higher rates compared to non-carriers (Fisher's Exact test: $P = 0.0095$).

Any phenotypes detected in this study could potentially be due to the psychosocial impact of raising a child with FXS. ANCOVA and linear regression analysis were performed with adjustment for raising a child with FXS in addition to other significant covariates. This adjustment had no effect on the statistical outcomes. Carriers of the premutation were then divided into two groups (those with and those without a child with FXS) and mean scores between the two groups were compared using ANCOVA. No score differences were detected for any mood or anxiety test.

Discussion

The purpose of this study was to examine phenotypes associated with mood and anxiety that may be associated with CGG repeat size or allele class status of the *FMR1* gene among younger adults, those who are at low risk for the clinical expression of FXTAS. Two primary strengths of this study were the relatively large sample size compared with other published studies and the ascertainment strategy that did not involve the fragile X-associated spectrum disorders. Specifically, we identified premutation carriers through families with a known diagnosis of a child with FXS, not because of their own symptoms, and we excluded subjects over the age of 50 in order to avoid the inclusion of premutation carriers with FXTAS. However, we must acknowledge that an ascertainment bias probably exists for any study of mood and anxiety phenotypes, since those who agree to participate in a research study may be less likely to have clinical mood and anxiety problems than those who do not. This would be true for both non-carriers and carriers of the premutation.

Our analyses did not detect any repeat length associations with social phobia, agoraphobia, or state or trait anxiety. However, we identified a subtle association between *FMR1* CGG repeat size and emotional phenotypes in males and females. Specifically, repeat length had a linear association with negative affect in males and females and with depression in males only (Tables 2 and 3). Though negative affect and depression represent two different factors, they are related. Increased negative affect is highly associated with depression along with decreased positive affect. However, no repeat length associations with positive affect were detected. In addition, negative affect is highly associated with anxiety, but no repeat length associations were detected in males or females with regard to anxiety. Other factors, such as age, race, and medication use, also contributed to the variation in emotional phenotype in our study, although we adjusted for these variables when examining the repeat length effects.

In a follow-up analysis of negative emotions from the PANAS, premutation males reported increased feelings of guilt and sadness compared to non-carriers while premutation females were at an increased risk of feeling fear and hostility (Tables 4 and 5). These contradictory results makes interpretation difficult, as one might expect the profile of negative emotions to be the same between males and females if it were related to the premutation effect. Further, an increased score for guilt could be expected among premutation females due to their risk of passing on an expanded allele which results in having a child with FXS. Therefore, the significant association between guilt scores and repeat length among males and not females is contrary to expectation.

These results support those of other recent studies that have reported emotional morbidity among premutation carriers (Dorn et al. 1994; Franke et al. 1998; Johnston et al. 2001; Hessl et al. 2005). Though the differences detected here are statistically significant at $P<0.05$, they are subtle and might not indicate a susceptibility to a clinical disorder. Indeed, all mean scores differences between female repeat length groups were of small effect size while the mean score difference noted among male repeat length groups was of medium effect size (Cohen 1992). Further, it is important to note that the mean scores for the premutation group were not within the diagnostic range for probable depression, probable social phobia, and panic disorder. However, males and females with the premutation did show higher rates of panic disorder and social phobia, respectively, and males with the premutation also showed higher rates of probable depression (Table 6).

In reporting results above, we used a significance level of 0.05 based on an unadjusted *P*-value, which is most likely too liberal due to multiple testing. However, the adjustment to the *P*-value to accommodate multiple testing is not straightforward: (1) the mood and

anxiety outcome variables are correlated among samples and (2) the two sets of analyses defining *FMR1* repeat length predictor in two ways (binary and continuous) are correlated. In an attempt to examine the effect of these influences on the *P*-values, we used the Cheverud–Nyholt estimate (Cheverud 2001; Nyholt 2004) to obtain an estimate of the number of effective tests given the correlation among the eight outcome measures. We found that the effective number of tests for the male and female samples would be 6.1 and 6.2, respectively. Using these results and applying the Bonferroni correction, significance at the 0.05 level would be indicated if the test outcome had an associated $P<0.0082$ (0.05/6.1) and $P<0.0081$ (0.05/6.2) for male and female analyses, respectively. With this adjustment, none of the results presented here remain statistically significant. Further adjustment to account for modeling each outcome measure twice, using repeat length as a continuous variable and repeat length as a categorical variable, would only increase the number of effective tests and lower the required *P*-value for statistical significance. Thus, all findings reported are only marginally significant and must be confirmed in independent studies. This and the small effect sizes, together, emphasize the subtlety of the phenotypic differences observed in this study.

A strength of this study was the use of measurements that provide scores related to severity of symptoms associated with psychiatric disorders, not just to the presence or absence of a clinical disorder. However, our use of self-report questionnaires provides only a `snapshot' of mental health at the time of testing, rather than a lifetime occurrence of a mental disorder. This is an important point, as most disorders, including depression and anxiety, tend to be episodic. In addition, self-report assumes the subjects retain insight into their mental health, irrespective of their situation on the day of testing.

In an effort to control for any effect of the psychosocial stress involved in raising a child with FXS, we performed additional analyses. We were not able to show that raising a child with FXS accounted for any of the mood and anxiety phenotype differences that we observed among women with and without the premutation. However, there are other factors potentially related to carrying the premutation (e.g., being a carrier and not having children, guilt of carrying a mutation, etc) that could influence mood and anxiety for which we could not account in our analyses.

The effect of age on emotional morbidity cannot be ignored, especially in the context of premutation carriers who are at risk for late onset FXTAS. Most often, clinical motor symptoms of FXTAS have an onset around mid 50s–60 years of age (Jacquemont et al. 2004). However, signs of cognitive impairment may precede motor symptoms (Grigsby et al. 2006). Our study population was limited to those ages 18–50 years. We suggest that the subtle emotional phenotypes reported here are most likely not due to the psychosocial stress of potentially having FXTAS. However, we cannot disregard the possibility that these phenotypes may be precursors to FXTAS. We tested this possibility by including age as a covariate in all analyses and did not detect any interaction between age and repeat length.

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References

Abrams L. From POF to POI: evolution of a term. National fragile X foundation quarterly. 2007; 29

Allen EG, He W, Yadav-Shah M, Sherman SL. A study of the distributional characteristics of FMR1 transcript levels in 238 individuals. Hum Genet. 2004; 114:439–447. doi:10.1007/s00439-004-1086-x. [PubMed: 14758538]

Allen EG, Sherman S, Abramowitz A, Leslie M, Novak G, Rusin M, et al. Examination of the effect of the polymorphic CGG repeat in the FMR1 gene on cognitive performance. Behav Genet. 2005; 35:435–445. doi:10.1007/s10519-005-2792-4. [PubMed: 15971024]

Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, et al. Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet B Neuropsychiatr Genet. 2003; 121:119–127. [PubMed: 12898586]

Bennetto L, Pennington BF, Porter D, Taylor AK, Hagerman RJ. Profile of cognitive functioning in women with the fragile X mutation. Neuropsychology. 2001; 15:290–299. doi:10.1037/0894-4105.15.2.290. [PubMed: 11324870]

Brown WT, Houck GE Jr, Jeziorowska A, Levinson FN, Ding X, Dobkin C, et al. Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. J Am Med Assoc. 1993; 270:1569–1575. doi:10.1001/jama.270.13.1569.

Cheverud JM. A simple correction for multiple comparisons in interval mapping genome scans. Heredity. 2001; 87:52–58. doi:10.1046/j.1365-2540.2001.00901.x. [PubMed: 11678987]

Cohen J. A power primer. Psychol Bull. 1992; 112:155–159. doi:10.1037/0033-2909.112.1.155. [PubMed: 19565683]

Cornish K, Kogan C, Turk J, Manly T, James N, Mills A, et al. The emerging fragile X premutation phenotype: evidence from the domain of social cognition. Brain Cogn. 2005; 57:53–60. doi:10.1016/j.bandc.2004.08.020. [PubMed: 15629215]

Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: human genome epidemiology review. Genet Med. 2001; 3:359–371. doi:10.1097/00125817-200109000-00006. [PubMed: 11545690]

Dorn MB, Mazzocco MM, Hagerman RJ. Behavioral and psychiatric disorders in adult male carriers of fragile X. J Am Acad Child Adolesc Psychiatry. 1994; 33:256–264. doi:10.1097/00004583-199402000-00015. [PubMed: 8150798]

Feng Y, Zhang F, Lokey LK, Chastain JL, Lakkis L, Eberhart D, et al. Translational suppression by trinucleotide repeat expansion at FMR1. Science. 1995; 268:731–734. doi:10.1126/science.7732383. [PubMed: 7732383]

Franke P, Leboyer M, Gansicke M, Weiffenbach O, Biancalana V, Cornillet-Lefebre P, et al. Genotype–phenotype relationship in female carriers of the premutation and full mutation of FMR-1. Psychiatry Res. 1998; 80:113–127. doi:10.1016/S0165-1781(98)00055-9. [PubMed: 9754690]

Garcia-Alegria E, Ibanez B, Minguez M, Poch M, Valiente A, Sanz-Parra A, et al. Analysis of FMR1 gene expression in female premutation carriers using robust segmented linear regression models. RNA (New York, NY). 2007; 13:756–762. doi:10.1261/rna.206307.

Grigsby J, Brega AG, Jacquemont S, Loesch DZ, Leehey MA, Goodrich GK, Hagerman RJ, Epstein J, Wilson R, Cogswell JB, Jardini T, Tassone F, Hagerman PJ. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). J Neurol Sci. 2006

Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. Am J Hum Genet. 2004; 74:805–816. doi:10.1086/386296. [PubMed: 15052536]

Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet B Neuropsychiatr Genet. 2005; 139:115–121. doi:10.1002/ajmg.b.30241. [PubMed: 16184602]

Hull C, Hagerman RJ. A study of the physical, behavioral, and medical phenotype, including anthropometric measures, of females with fragile X syndrome. Am J Dis Child. 1960; 147:1236–1241. 1993. [PubMed: 8237919]

Hunter J, Abramowitz A, Rusin M, Sherman S. Neuropsychological and neurobehavioral phenotypes among FMR1 premutation allele carriers: a review of current literature. Behav Genet. submission.

Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. J Am Med Assoc. 2004; 291:460–469. doi:10.1001/jama.291.4.460.

Johnston C, Eliez S, Dyer-Friedman J, Hessl D, Glaser B, Blasey C, et al. Neurobehavioral phenotype in carriers of the fragile X premutation. Am J Med Genet. 2001; 103:314–319. doi:10.1002/ajmg.1561. [PubMed: 11746012]

Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet. 2001; 10:1449–1454. doi:10.1093/hmg/10.14.1449. [PubMed: 11448936]

Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, et al. Technical standards and guidelines for fragile X: the first of a series of disease-specific supplements to the standards and guidelines for clinical genetics laboratories of the American college of medical genetics. Quality assurance subcommittee of the laboratory practice committee. Genet Med. 2001; 3:200–205. doi:10.1097/00125817-200105000-00010. [PubMed: 11388762]

Meadows KL, Pettay D, Newman J, Hersey J, Ashley AE, Sherman SL. Survey of the fragile X syndrome and the fragile X E syndrome in a special education needs population. Am J Med Genet. 1996; 64:428–433. doi:10.1002/(SICI)1096-8628(19960809)64:2<428::AID-AJMG39>3.0.CO;2-F. [PubMed: 8844098]

Nolin SL, Brown WT, Glicksman A, Houck GE Jr, Gargano AD, Sullivan A, et al. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. Am J Hum Genet. 2003; 72:454–464. doi:10.1086/367713. [PubMed: 12529854]

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004; 74:765–769. doi:10.1086/383251. [PubMed: 14997420]

Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, et al. Absence of expression of the FMR-1 gene in fragile X syndrome. Cell. 1991; 66:817–822. doi:10.1016/0092-8674(91)90125-I. [PubMed: 1878973]

Primerano B, Tassone F, Hagerman RJ, Hagerman P, Amaldi F, Bagni C. Reduced FMR1 mRNA translation efficiency in fragile X patients with premutations. RNA (New York, NY). 2002; 8:1482–1488.

Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. Appl Psychol Meas. 1977; 1:385–401. doi:10.1177/014662167700100306.

Reiss AL, Dant CC. The behavioral neurogenetics of fragile X syndrome: analyzing gene-brain-behavior relationships in child developmental psychopathologies. Dev Psychopathol. 2003; 15:927–968. doi:10.1017/S0954579403000464. [PubMed: 14984133]

Reiss AL, Freund L, Abrams MT, Boehm C, Kazazian H. Neurobehavioral effects of the fragile X premutation in adult women: a controlled study. Am J Hum Genet. 1993; 52:884–894. [PubMed: 8488838]

Sherman S, Pletcher BA, Driscoll DA. Fragile X syndrome: diagnostic and carrier testing. Genet Med. 2005; 7:584–587. doi:10.1097/01.GIM.0000182468.22666.dd. [PubMed: 16247297]

Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Gen. 2000; 97:189–194. doi:10.1002/1096-8628(200023)97:3<189::AID-AJMG1036>3.0.CO;2-J.

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN. Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. Am J Hum Genet. 1993; 53:1217–1228. [PubMed: 7902673]

Sobesky WE, Taylor AK, Pennington BF, Bennetto L, Porter D, Riddle J, et al. Molecular/clinical correlations in females with fragile X. Am J Med Genet. 1996; 64:340–345. doi:10.1002/(SICI)1096-8628(19960809)64:2<340::AID-AJMG21>3.0.CO;2-E. [PubMed: 8844077]

Spielberger, C. Manual for the state-trait anxiety inventory for adults (form Y). Mind Garden, Redwood City, CA: 1983.

Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, et al. DNA methylation represses FMR-1 transcription in fragile X syndrome. Hum Mol Genet. 1992; 1:397–400. doi:10.1093/hmg/1.6.397. [PubMed: 1301913]

Tassone F, Hagerman PJ. Expression of the FMR1 gene. Cytogenet Genome Res. 2003; 100:124–128. doi:10.1159/000072846. [PubMed: 14526172]

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet. 2000a; 66:6–15. doi:10.1086/302720. [PubMed: 10631132]

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, et al. Clinical involvement and protein expression in individuals with the FMR1 premutation. Am J Med Genet. 2000b; 91:144–152. doi:10.1002/(SICI)1096-8628(20000313)91:2<144::AID-AJMG14>3.0.CO;2-V. [PubMed: 10748416]

Thompson NM, Gulley ML, Rogeness GA, Clayton RJ, Johnson C, Hazelton B, et al. Neurobehavioral characteristics of CGG amplification status in fragile X females. Am J Med Genet. 1994; 54:378–383. doi:10.1002/ajmg.1320540418. [PubMed: 7726212]

Turner, D.; Beidel, SM.; C. Dancu, C. V Social phobia and anxiety inventory: manual. Multi-Health Systems, Inc; Toronto, Ont: 1996.

Watson, D.; Clark, LA. Manual for the positive and negative affect schedule (expanded form). University of Iowa, Iowa City, IA: 1994.

Wechsler, D. Wechsler adult intelligence scale. 3rd edition manual. The Psychological Corporation; San Antonio: 1997.

Welt CK. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin Endocrinol (Oxf). 2007

Table 1

Demographic data of study male and female study participants stratified by *FMR1* repeat length group

Gender	Repeat length group	*N*	Mean age (SD)	Ethnicity % Caucasian/Asian	Education % college or higher	Income % $50,000 or higher	Mean FSIQ (SD)	Ascertainment % GP	Anxiety/depression medication use % on meds
Males	All	119	35.8 (9.4)	78.2[a,b]	77.3	67.3	110.4 (14.6)	48.7[c]	5.0
	NC	61	36.2 (8.9)	82.0	83.3	69.0	110.2 (12.7)	44.3	8.2
	IM	32	33.6 (10.3)	59.4	78.1	64.5	110.9 (18.2)	96.9	3.1
	PM	26	37.8 (9.3)	92.3	61.5	66.7	110.2 (14.5)	0.0	0.0
Females	All	446	35.1 (9.5)[b]	76.4[c,d]	86.1	61.4[c]	107.9 (13.2)	38.6[c]	14.3[c]
	NC	97	32.7 (10.0)	58.8	92.8	56.3	109.0 (13.8)	82.5	7.2
	IM	94	32.0 (11.1)	59.6	87.2	47.8	106.6 (15.2)	89.4	8.6
	PM	255	37.1 (8.2)	89.4	83.1	68.4	108.0 (12.1)	3.1	18.8

NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers; SD = standard deviation; FSIQ = full scale intelligence quotient; GP = general population

[a] $P < 0.05$ for comparison among repeat groups

[b] Male participants consisted of 78.2% Caucasian, 0% Asian, 19.3% African American, and 2.5% Hispanic subjects

[c] $P < 0.005$ for comparison among repeat groups

[d] Female participants consisted of 75.5% Caucasian, 0.9% Asian, 18.4% African American, 3.4% Hispanic, and 1.8% `other' ethinicity subjects

Table 2

Results from the general linear model using *FMR1* repeat length as the main predictor of neurobehavior phenotypes

Gender	Measure	Subscale	*P* estimates	*P* value
Males	CES-D	Depression	0.2601	0.03
	STAI	State anxiety	0.0800	0.40
		Trait anxiety	0.1292	0.30
	PANAS	Negative affect	0.1914	0.04
		Positive affect	−0.2360	0.08
	SPAI	Social phobia	0.0837	0.49
		Agoraphobia	0.1462	0.22
		“Pure” social phobia	0.0162	0.88
Females	CES-D	Depression	0.0323	0.45
	STAI	State anxiety	−0.0333	0.72
		Trait anxiety	0.1047	0.15
	PANAS	Negative affect	0.0883	0.04
		Positive affect	−0.0353	0.64
	SPAI	Social phobia	−0.0319	0.64
		Agoraphobia	0.0951	0.08
		“Pure” social phobia	−0.0034	0.96

CES-D = Centers for Epidemiologic Studies Depression Scale; STAI = State-Trait Anxiety Inventory; PANAS = Positive and Negative Affect Schedule; SPAI = Social Phobia and Anxiety Inventory

Table 3

Results from the general linear model results using indicator variables to compare *FMR1* repeat length groups as the main predictors of neurobehavior phenotypes

Gender	Measure	Subscale	β estimates	*P* value	Adjusted group means
Males	CES-D	Depression	NC: ref	NC: ref	NC: 2.55
			IM: 0.20	IM: 0.11	IM: 3.17
			PM: 0.25	PM: 0.08	PM: 3.36
	STAI	State anxiety	NC: ref	NC: ref	NC: 3.4
			IM: 0.07	IM: 0.72	IM: 3.5
			PM: 0.03	PM: 0.76	PM: 3.4
		Trait anxiety	NC: ref	NC: ref	NC: 3.45
			IM: −0.02	IM: 0.93	IM: 3.44
			PM: 0.13	PM: 0.39	PM: 3.53
	PANAS	Negative affect	NC: ref	NC: ref	NC: 2.87
			IM: 0.07	IM: 0.55	IM: 2.92
			PM: 0.17	PM: 0.19	PM: 3.01
		Positive affect	NC: ref	NC: ref	NC: 37.35
			IM: −0.07	IM: 0.48	IM: 36.44
			PM: −0.30	PM: 0.07	PM: 32.96
	SPAI	Social phobia	NC: ref	NC: ref	NC: 6.99
			IM: 0.10	IM: 0.31	IM: 7.57
			PM: 0.09	PM: 0.45	PM: 7.53
		Agoraphobia	NC: ref	NC: ref	NC: 3.06
			IM: 0.08	IM: 0.43	IM: 3.35
			PM: 0.17	PM: 0.15	PM: 3.76
		“Pure” social phobia	NC: ref	NC: ref	NC: 6.09
			IM: 0.13	IM: 0.86	IM: 6.79
			PM: 0.02	PM: 0.24	PM: 6.21
Females	CES-D	Depression	NC: ref	NC: ref	NC: 3.01
			IM: 0.02	IM: 0.82	IM: 3.09
			PM: 0.02	PM: 0.71	PM: 3.07
	STAI	State anxiety	NC: ref	NC: ref	NC: 3.5
			IM: −0.03	IM: 0.79	IM: 3.5
			PM: −0.08	PM: 0.48	PM: 3.4
		Trait anxiety	NC: ref	NC: ref	NC: 3.53
			IM: −0.03	IM: 0.71	IM: 3.51
			PM: 0.11	PM: 0.27	PM: 3.59
	PANAS	Negative affect	NC: ref	NC: ref	NC: 2.88 *
			IM: 0.01	IM: 0.89	IM: 2.89
			PM: 0.22	PM: 0.02	PM: 3.04 *
		Positive affect	NC: ref	NC: ref	NC: 35.74
			IM: −0.04	IM: 0.53	IM: 35.11

Gender	Measure	Subscale	β estimates	*P* value	Adjusted group means
			PM: −0.01	PM: 0.95	PM: 35.65
	SPAI	Social phobia	NC: ref	NC: ref	NC: 7.65
			IM: 0.07	IM: 0.19	IM: 8.10
			PM: 0.01	PM: 0.94	PM: 7.68
		Agoraphobia	NC: ref	NC: ref	NC: 3.91
			IM: 0.10	IM: 0.09	IM: 4.31
			PM: −0.06	PM: 0.40	PM: 3.71
		“Pure” social phobia	NC: ref	NC: ref	NC: 6.35
			IM: 0.05	IM: 0.34	IM: 6.71
			PM: 0.02	PM: 0.75	PM: 6.48

ref = reference group; CES-D = Centers for Epidemiologic Studies Depression Scale; STAI = State-Trait Anxiety Inventory; PANAS = Positive and Negative Affect Schedule; SPAI = Social Phobia and Anxiety Inventory; NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers

Table 4

Post hoc analysis to further explore negative emotion subscale scores from the Positive and Negative Affect Schedule. Results are obtained from the general linear model results using *FMR1* repeat length as the main predictor

Gender	Subscale	β estimates	*P* value
Males	Fear	0.1468	0.23
	Sadness	0.1981	0.03
	Guilt	0.2901	0.01
	Hostility	0.1286	0.28
Females	Fear	0.0112	0.87
	Sadness	0.0013	0.98
	Guilt	0.0429	0.55
	Hostility	0.0566	0.16

Table 5

General linear model results using indicator variables to compare *FMR1* repeat length groups as the main predictors. Follow-up on specific negative emotion subscale scores from the Positive and Negative Affect Schedule

Gender	Subscale	β estimates	*P* value	Adjusted group means
Males	Fear	NC: ref	NC: ref	NC: 10.62
		IM: −0.04	IM: 0.18	IM: 10.24
		PM: 0.18	PM: 0.66	PM: 12.35
	Sadness	NC: ref	NC: ref	NC: 8.96
		IM: 0.12	IM: 0.33	IM: 10.16
		PM: 0.22	PM: 0.08	PM: 11.29
	Guilt	NC: ref	NC: ref	NC: 9.01[a,b]
		IM: 0.23	IM: 0.04	IM: 11.33[a]
		PM: 0.30	PM: 0.03	PM: 12.20[b]
	Hostility	NC: ref	NC: ref	NC: 11.68
		IM: −0.01	IM: 0.99	IM: 11.67
		PM: 0.16	PM: 0.23	PM: 13.52
Females	Fear	NC: ref	NC: ref	NC: 10.62[b]
		IM: −0.01	IM: 0.93	IM: 10.57[a]
		PM: 0.18	PM: 0.05	PM: 12.21[a,b]
	Sadness	NC: ref	NC: ref	NC: 9.99
		IM: 0.01	IM: 0.41	IM: 10.12
		PM: 0.07	PM: 0.83	PM: 10.63
	Guilt	NC: ref	NC: ref	NC: 10.19
		IM: −0.01	IM: 0.98	IM: 10.17
		PM: 0.13	PM: 0.11	PM: 11.45
	Hostility	NC: ref	NC: ref	NC: 11.28[b]
		IM: 0.02	IM: 0.70	IM: 11.53
		PM: 0.16	PM: 0.05	PM: 12.85[b]

NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers; ref = reference group

[a,b]Group mean scores are different at the $P = 0.05$ level

Table 6

Clinical diagnoses determined from the Center for Epidemiologic Studies Depression Scale (CES-D) and social phobia and panic disorder scales of the Social Phobia and Anxiety Inventory (SPAI) by gender and repeat length group

Gender	Repeat Length Group	CES-D probable depression	SPAI probable social phobia	SPAI probable panic disorder
Males	All, $n = 119$	22 (18.5%)[a]	14 (11.8%)	3 (2.5%)[a]
	NC, $n = 61$	5 (8.2%)	6 (9.8%)	0 (0.0%)
	IM, $n = 32$	10 (31.3%)	3 (9.4%)	0 (0.0%)
	PM, $n = 26$	7 (26.9%)	5 (19.2%)	3 (11.5%)
Females	All, $n = 446$[b]	115 (25.8%)	69 (15.5%)[c]	27 (6.1%)
	NC, $n = 97$	23 (23.7%)	5 (5.2%)	3 (3.1%)
	IM, $n = 94$	25 (26.6%)	11 (11.7%)	6 (6.4%)
	PM, $n = 255$	67 (26.3%)	53 (20.9%)	18 (7.1%)

NC = non-carriers; IM = intermediate allele carriers; PM = premutation allele carriers

[a] Fisher's Exact test, $P < 0.05$

[b] SPAI scores were unavailable for 2 female participants, 1 PM and 1 NC

[c] Fisher's Exact test, $P < 0.005$